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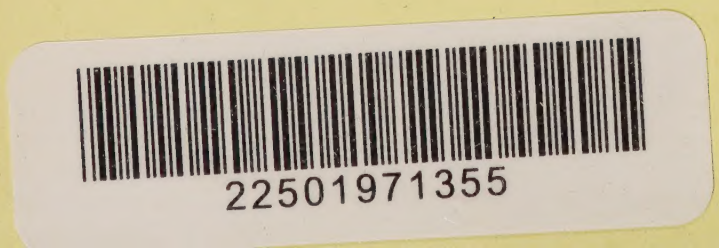

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PUBLIC HEALTH.

REPORTS

OF

THE MEDICAL OFFICER OF THE PRIVY COUNCIL AND LOCAL GOVERNMENT BOARD.

NEW SERIES, No. III.

REPORT TO THE LORDS OF THE COUNCIL ON SCIENTIFIC INVESTIGATIONS, MADE UNDER THEIR DIRECTION, IN AID OF PATHOLOGY AND MEDICINE.

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MEDICAL OFFICER'S REPORT.

To the Lords of Her Majesty's Most Honorable Privy Council.

My Lords,

In continuation of former reports, referring to Scientific Investigations made under your Lordships in this Department, I have now the honor of laying before your Lordships the subjoined five papers, and would beg leave to prefix to them a few introductory words, first, with regard to the branch of work to which the papers belong, and then with regard to the papers respectively.

The purpose of the Scientific Investigations which are made in this Department is to contribute in a particular way to the progress of the Art of Medicine.

The sorts of study by which the Medical Profession hopes to extend its means of usefulness to mankind, whether as regards the cure of individual cases of disease, or as regards those large acts of preventive medicine which are of interest to masses of population, may broadly be distinguished as two. On the one hand, there are studies which every member of the Medical Profession (more or less according to his circumstances) has both opportunity and inducement to cultivate at least for his own use, and generally for that of others, by intelligent observation and record of the facts which come before him in his daily practical work, and by scientific generalisations from such experience; and I need hardly observe that, from studies of that sort, which are a life-long happiness to innumerable medical practitioners, the aggregate efficiency of the Profession is always in course of increase. On the other hand there are extremely important studies, for which the ordinary professional practice supplies neither opportunity nor immediate stimulus; studies, namely, of elaborate and purely scientific research in aid of the development of medical knowledge; studies never immediately convertible to pecuniary profit, but perhaps, on the contrary, involving heavy cost; studies, too, which from their nature cannot promise rapid results, nor be conducted in fragments of leisure, but require systematic and continuous labor extending over long periods of time.

It cannot be expected that studies of this latter sort, even where of the highest eventual importance, should generally be cultivated to any adequate extent by private medical investigators; and the distinctive intention of the Scientific Investigations which are conducted under your Lordships' auspices has been to supplement in that direction the ordinary resources of private medical observation. It is of the essence of the case that the work does not pretend to immediate popular application, but addresses itself

primarily to the deeper scientific requirements of the Medical Profession, and is therefore in an extreme degree technical. It is also of the essence of the case that, in common with everything which is tentative, the work may sometimes have its turn of temporary unsuccess and disappointment; and that therefore its results (as indeed the results of every scrupulous scientific investigation) are less suited to be measured from year to year than in terms of several years taken together.

The work which is at present in progress is an exceedingly large and various study, having many divisions and subdivisions of its own, and sometimes (as will be seen) involving collateral inquiry, but nevertheless, in its intention, quite definite and single. It aims to be a systematic study of the intimate pathology of the *Morbid Infections*, acute and chronic.

Such study in regard of any one infection has of course several lines of investigation: for, on the one hand, the infective agent has to be identified; and, on the other hand, the successive changes which it effects in the living body have, from beginning to end, to be made intelligible. Then, scientifically speaking, no morbid change in the body can be stated in intelligible terms except with exact comparative reference to health as a standard; and in the present case the things and processes as to which the questions of comparison arise, and which belong to the subtlest subject-matters of physiological research, are hitherto, in a large part of the field, but very imperfectly known as to their normal standards: so that, to this large extent, an indispensable preliminary to progress in the pathological study is at least to verify and complete the required physiological standards of comparison, and in not a few cases to invent new methods of procedure by which observations so subtle may be conducted.

Without pretending to prejudge exactly what may be the eventual application of each addition made to scientific knowledge, I may observe in general terms that some physiological and pathological work seems rather to connect itself with the hopes of Preventive, other rather with the hopes of Curative, Medicine. Every process of disease admits of being contemplated and investigated from either of two opposite points of view: either, namely, from the side of the morbific cause, and with particular reference to the initiatory acts of disease; or from the side of the final morbid effect, and with particular reference to the modes of operation by which disease tends to destroy life. Researches in the former sense may peculiarly be expected to increase our powers of preventing disease, and researches in the latter sense to suggest improvements in therapeutical practice.

It is chiefly from the former of those points of view that Professor Sanderson has for some years been working under your Lordships in one large part of the Scientific Investigations; in which

division of the field he has of late been very ably seconded by Assistant-Professor Dr. Klein, and on the present occasion also presents a contribution of much interest by Dr. Creighton.

Of the annexed papers the first two, respectively by Dr. Sanderson and Dr. Klein, relate to the intimate ætiology of the acute infective diseases. Ten years ago we had not even a beginning of any true insight into the respective *Contagia* which excite those diseases; and considering the large and lasting interest which exact studies in this field of scientific research must have for the human race, I think the fact noteworthy that the first of such studies were instituted, and the first steps of discovery made, with reference to a contagious fever of horned cattle. I refer, namely, to the researches which were made under Her Majesty's Government in 1865 in aid of the then Cattle Plague Commission: when Dr. Beale, working at the microscopy of the disease, drew attention to the swarms of extremely minute particles which he found universally present in the textures and juices of the animals, and which he believed to be the contagium of the disease; and when Dr. Sanderson, working at the matter from a different point of view, succeeded in showing experimentally that the true contagium admits of being physically distinguished in the animal juices which contain it, and of being so separated from them as to leave them without infective power. In the next succeeding years, the writings (to which I have on former occasions adverted) of Dr. Hallier, Professor of Botany in Jena, brought under animated discussion, as a branch of micro-phytology, the nature and mode of origin of contagium-particles in a great variety of diseases, human and brute: new experimental knowledge of several contagia was set forth in the writings (to which also I have before referred) of Professor Chauveau, of the Veterinary School of Lyons; and in 1870 I had the honor of presenting Dr. Sanderson's first report of researches made in the matter under your Lordships' direction. At that time, general conclusions already seemed justified; first, that the characteristic shaped elements, which the microscope had shown abounding in various infective products, are self-multiplying organic forms, not congeneric with the animal body in which they are found, but apparently of the lowest vegetable kind; and, secondly, that such living organisms are probably the essence, or an inseparable part of the essence, of all the contagia of disease. The study of morbid contagion was thus brought into seeming affinity with that which had for some years before been made by Professor Schröder and M. Pasteur in the ordinary processes of fermentation and putrefaction; and there began to become faintly visible to us a vast destructive laboratory of Nature, wherein the diseases which are most fatal to animal life, and the changes to which dead organic matter is passively liable, appear bound together by what must at least be called a very close analogy of causation. This view of the matter has since then become greatly more distinct in consequence of investigations made under your Lordships by Dr. Sanderson, particularly in 1871 and

1872, with reference to the common septic contagium or ferment;* for in that ferment, particulate as above described, there seems now to be identified a force which, acting disintegratively upon organic matter alike whether dead or living, can on the one hand initiate putrefaction of what is dead, and on the other hand initiate febrile and inflammatory processes in what is living.

Continuation of this line of study in regard of acute infections of the living body is represented in the first two of the appended papers.

In the first paper Dr. Sanderson brings down to the present time an account of the *Microphytes of Contagion;* setting forth more particularly such positive knowledge as has yet been obtained with regard to the respective contagia and respective morbid processes of diphtheria, erysipelas, relapsing fever, and (though but rarely a human disease) the splenic fever or "milz-brand" of veterinary practice.

The second paper represents a contribution to the growing modern doctrine of contagion, in an exposition by Dr. Klein of the intimate nature of the local changes which characterise the acute zymotic disease known as *Variola Ovina* or *Sheep-Pox.* Dr. Klein has been able to identify the contagium-particles of that infectious fever as definite microphytes, growing and fructifying with vast rapidity in the canals and tissues of the infected skin: the woodcuts of his annexed paper show the process to have been observed by him with a completeness not yet, I believe, attained in regard of any other such case: and these results of his, while they complete as regards the special disease in question the broad pathological outline which previous inductions had rendered probable, must also, I think, be regarded as tending very importantly to confirm, while they illustrate, the general doctrine of the vitality of contagia.

The third of the annexed papers, also by Dr. Klein, and founded on work of his which has been in progress for more than two years, continues a line of research already much worked by Dr. Sanderson, with regard to the most important of all chronic infective processes: that, namely, which is at the root of the so-called *Tubercular Diseases* of the animal body, and which has its highest practical interest in relation to the pulmonary phthisis of the human subject. In my Report of 1868, I had to mention to your Lordships an infinitely interesting light of new knowledge with regard to tubercular disease, in the discovery which M. Villemin had recently made, and which other observers had immediately confirmed, of its being inoculable from subject to subject. During the years which have since passed, further and very striking illustration of the communicability of tubercle has

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* For convenience, in speaking of this morbific influence, I use the singular number, but have no intention of implying that ordinary putrefactive changes have only one ferment which can be considered habitual to them.

been given by the results of experimental feeding with the milk of living tubercular animals, or with bits of diseased texture from such animals when dead; it having been found that in the subjects of such experiments the contagium penetrates by the absorbent organs of the alimentary canal, and so diffuses from them its influence to other organs of the body as to produce general tubercular disease.* With my Reports of 1868 and 1869 I submitted to your Lordships important papers by Dr. Sanderson on the studies which had then been made of tubercle in its infective relations; and the paper by Dr. Klein which I now submit gives a second series of results in the same subject-matter. It has from the first been a necessary part of the researches into the processes of tubercular infection in the animal body, that very exact study should be made of the intimate anatomical conditions which determine the sequence of its phenomena and make different organs differently susceptible of it. In the earlier researches Dr. Sanderson found himself obliged for this reason to make much anatomical study of the lymphatic glands; and in the later researches it was an indispensable first need that the knowledge of the lymphatic system should be completed by an exhaustive investigation of the radicles and gland-rudiments of that system in at least the serous membranes and the lungs. Dr. Klein's fulfilment of that large preliminary task has added very valuably to previous knowledge of the normal anatomy; but in the accompanying paper, which essentially relates to the course of tubercular infection in the organs referred to, these results of his two years' work are only given with such amount of detail as may make the morbid anatomy quite intelligible.†

The broad results of modern discovery in regard of ordinary tubercular disease tend to represent it as a chronic locally-originated zymotic process, which, starting under certain conditions in one first spot of the (predisposed) animal body, advances by successive steps in definite anatomical lines to infect the entire system: a process, which by means of its characteristic products is inoculable from part to part, and from subject to subject; but of which in certain other cases the locally originating cause or contagium appears to be the common septic ferment, or a ferment not yet separable from the septic.‡ This

* In this connexion it deserves notice that within the last few weeks Dr. Hermann Weber has brought before the Clinical Society some facts of a strikingly suggestive kind as to the possibility of tubercular infection being freely communicated to women through their conjugal relations with men who have pulmonary phthisis in a quiescent or very chronic state.

† I may note that these studies of Dr. Klein's, and also that relating to Sheep-Pox, were completed at a time when my former series of reports had come to an end and the plan of the present series was not yet settled; and that, as it was desirable not to delay scientific publication of the results, the papers were, with their Lordships sanction, communicated at once to the Royal Society.

‡ That the acute tuberculisation of organs is preceded by an infection of the blood is made in the highest degree probable by a consideration of the circumstances of cases: but, as to the source from which this infection would first have come, evidence enough for judgment is not yet given.

being the tendency of recent knowledge in regard of tubercle, evidently there must arise from it very important suggestions (on which I need not here dwell) both as to further like studies of the same disease, and as to tentative applications of the knowledge in the province of preventive medicine; and suggestion also arises with regard to a collateral interest scarcely less important than that of tubercle.

In view, namely, of these results of the modern investigation of tubercle, question naturally arises whether analogous investigation of *Cancer* might not be equally successful. The analogy between the two sorts of disease must of course not be overstrained, nor be construed into any prejudgment of facts; but subject to this condition it will, I think, be generally admitted that cancer and tubercle are morbid processes so nearly parallel as probably to throw light on one another, and that methods of investigation which have led to an immensely improved knowledge of the one disease can hardly not lead to instructive results concerning the other. Careful consideration has therefore been given to the possibility of investigating more exactly than hitherto the chronic self-propagative process which is at the root of the diseases called cancerous; and a first preparatory step under your Lordships for the purpose of such an investigation is represented in the fourth of the annexed papers. In it, namely, is the statement of an interesting study which Dr. Creighton has made, and of which the results are there given, with regard to the earlier signs of cancerous infection in the ultimate anatomical elements of the secondarily infected organs.

The fifth of the annexed papers belongs to another sphere of study than the four preceding. It contains, so far as yet ripe for publication, the results of a very important and very laborious research, on which Dr. Thudichum has been engaged for more than two years, in introduction to the *Chemical Pathology of the Nervous System.* During the years 1870–2, Dr. Thudichum, in accordance with the general scheme of work which I explained in my 12th Report, had been investigating the chemistry of typhus; but after following as far as then seemed possible the chemical phenomena of that disease, had found that the results of his work could not properly be deemed more than provisional, and that, under the circumstances as they then stood, direct attainment of final results could not be expected. The difficulty was of a sort to which I have already adverted: it consisted in the want of normal chemical standards by which to measure the morbid declensions in typhus. Especially a leading fact in typhus, one which may be in intimate relation to the killing-power of the disease, and which it is on all accounts necessary to have as completely as possible understood, is the fact of the action on the nervous system; and clearly no true chemical knowledge of that morbid action would be possible, till greatly improved knowledge of the normal brain-chemistry should be

supplied. Accordingly this task was taken in hand; and of course not with exclusive reference to the one disease which gave immediate occasion to it, but in the hope of eventually obtaining results which should be applicable to all cerebral pathology. I need hardly observe that a research of such interest—an endeavour so to unfold the very complex chemistry of the normal brain as to make the abnormal processes chemically intelligible, must, as regards the interest and importance of its object, rank highly among the exact studies by which physiology hopes to give light to Medicine; but I would particularly note that while certainly it is among the most interesting and important of such endeavours, so it certainly must be counted among the most difficult; and I therefore refer with particular satisfaction to the very remarkable evidences of success which Dr. Thudichum is already able to show.

In addition to bringing before your Lordships the accompanying complete papers, I am able to state that much other and equally important work is at present in progress. Especially I may mention that Dr. Sanderson, who for the last three years has been continuously engaged in studies of the infective processes of common fever and common inflammation, will, I believe, early next year have an instalment of valuable results ready for publication with regard to these investigations*; and that Dr. Klein, who has recently been investigating the minute anatomy of enteric fever, and seems already to have succeeded in identifying for the first time the contagium-particles of that extremely important disease, will also, I believe, early next year have his results in this matter sufficiently elaborated for publication.

In closing my present Report, and with the interest which I must of course feel in whatever tends to strengthen the scientific resources of the Medical Profession, I would beg leave respectfully to congratulate your Lordships on the progress of this work which is advancing under your authority, and to express the gratification with which from time to time I find myself privileged to bring its results before you. Equally to your Lordships who have instituted the work, and to the men of science who under your Lordships are instrumental in conducting it, the reflection must, I think, be satisfactory, that, so far as the Investigations fulfil their primary aim of giving aid and

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* Here I may note that two years ago, in the interval between my two series of reports, Dr. Sanderson reported to me some first results which he had then obtained in regard of the infective products of inflammation; and that with their Lordships' sanction (in order not to delay scientific publication of those results) his paper was communicated to the Royal Medical and Chirurgical Society, who published it in the 56th volume of their Transactions. At Dr. Sanderson's suggestion I do not now annex that paper in its original form, but leave its matter to be incorporated by him in a future more comprehensive report.

stimulus to the growth of Medical Science in this one country, their success is contributory to knowledges which are of universal human concern, and which it has therefore ever been the aspiration and the pride of each civilized people to augment for the common good of mankind.

I have the honor to be,
My Lords,
Your obedient servant,
John Simon.

Medical Department;
July 30th, 1874.

APPENDIX.

No. 1.—Report on Recent Researches on the Pathology of the Infective Processes, by J. Burdon Sanderson.

Since the publication of my former contribution on this subject,* it has engaged a very large share of the attention of scientific pathologists in all parts of the world. As some of the many investigations which have been made have led to results of great importance, I propose to give an account of them. Before doing so, however, it is probably desirable to recapitulate very shortly the conclusions formerly arrived at.

The paper to which I have referred was divided into two parts; in the first an attempt was made to support the position which had then been recently advanced by my friend Professor Chauveau, that in all diseases communicable from person to person, whether by direct or indirect contagion, the infecting agent or *contagium* consists of particles of extreme minuteness, with respect to which it can be asserted that the material of which they are composed is *not* soluble in animal liquids, that it is *not* volatile at ordinary temperatures, and that it possesses a specific gravity very slightly different from that of the liquid in which the particles are suspended.

This conclusion, which was expressed in the sentence, "All contagia are probably particulate," has now, either in the same terms, or in others equivalent, been very generally accepted by pathologists. It was not understood to carry with it any meaning beyond that which is conveyed by the words as interpreted in their strictest sense, and left the question of the form of the hypothetical particles, as well as that of their origin and destiny, entirely open for future investigation. In support of it I set forth the results of Chauveau's now well-known experiments relating to the contagious products of sheep-pox, glanders, variola, and vaccine, many of which I had shortly before had the opportunity of witnessing at Lyons. Those of them which related to vaccine I repeated, modifying them in such a way as to give them the utmost obtainable exactness.

In the second part of the same paper I pursued the subject in an entirely different direction. Starting from the proposition already referred to, that in all probability contagia are particulate, and that the particles are possessed of the properties I had assigned to them, I endeavoured to show that this assumption, when applied to the so-called specific diseases, almost necessarily carried with it the admission of the doctrine of *contagium vivum*, the facts relating to the propagation of contagious diseases through the atmosphere being of such a nature, that supposing such propagation to consist in the conveyance from the diseased to the healthy body of the infective particles, those particles must enjoy endowments not known to exist otherwise than in association with life and organisation.

In connexion with this subject I gave an account of the then recently published researches of Professor Hallier, of Jena, researches undertaken in the hope of demonstrating the identity between the specificity of disease and that of organic forms. As it would have been impossible to have examined into the whole mass of investigations undertaken in this field by that indefatigable mycologist, I confined myself to a careful

* "On the Intimate Pathology of Contagion. Thirteenth Report of the Medical Officer of the Privy Council."

analysis of the experimental demonstration he professed to give with respect to two important contagia (viz., those of cholera and sheep-pox), of the existence of a definite and constant relation between the human or animal disease on the one hand, and a corresponding parasitic disease of plants used as food on the other. As regards the connexion of cholera with the parasitic disease of the rice plant, supposed by Hallier to be prevalent in the rice-growing districts of Bengal, I showed that there was not even *primâ facie* evidence in support of the author's theory. I also showed that his supposition as to the dependence of sheep-pox on a specific disease of the grass on which the affected animals are pastured, had no foundation.

The following papers, in which I propose to give an account of the present state of our knowledge as to the pathology of the infective processes in erysipelas, splenic fever, diphtheria, and relapsing fever, are intended to form a sequel to my former Report.

There are two relations in which the life of the lowest plants may be supposed to stand to the processes of disease; the one is a relation between *specific* diseases and specific organic vegetable forms; the other between what may be called the *common* processes of disease and those elementary organisms often spoken of as bacteria, which spring up and thrive as if spontaneously, wherever moisture and nitrogen in an available state of combination are present.

These two relations are entirely distinct from each other. They involve different questions and require different modes of investigation. I therefore propose to divide the account to be given in the following pages of some of the most important recent researches relating to the vegetative phenomena of disease, into two parts; the first relating to those diseased processes, mostly of inflammatory origin, in which the ordinary microphytes, associated with the septic transformation of nitrogenous matter, appear to play an important part; the second relating to one or two instances in which the search for botanical characteristics appears to have been successful, the specificity of the disease being represented by a vegetation of recognisable peculiarities of form and development.

Part I.

Nomenclature of septic microphytes.

The air we breathe with its particles of dust, and the water we drink, whether in the ordinary sense pure or impure, contain organisms of extreme minuteness and simplicity of form, which are now commonly spoken of as bacteria. For these organisms, or rather for the group of organic forms which they represent, I proposed in my former paper the term "microzymes," the only name by which they were then and are still known to naturalists (*Schizomycetes*), being too long for common use. Of the legion of observers who have contributed to the literature of the subject during the last few years, most have used Hallier's word *micrococcus*. The surgical pathologists, on the other hand, have devised words for themselves. Klebs, in his book on gunshot wounds, introduced the term *microsporon septicum*.* Hueter in his studies on surgical fever and other secondary traumatic affections has used the word "monads;"† and lastly Billroth has entitled the massive folio which he has lately published on the same subject, *Coccobacteria septica*.‡

* Klebs, Beiträge zur pathologischen Anatomie der Schusswunden, 4to., pp. 133, Leipzig, 1872.

† Hueter, Ueber die chirurgische Behandlung der Wundfieber bei Schusswunden. Volkmann's klinische Vorträge, No. 22, Leipzig, 1871.—Zur Aetiologie und Therapie der metastasirenden Pyaemien. Deutsche Zeitschr. f. Chirurgie, 1872, p. 91.

‡ Billroth, Untersuchungen über die Vegetationsformen von Coccobacteria septica, fol., pp. 244. Berlin, 1874.

In the existing confusion of terms, it is probably best to continue to employ those words which express most simply the forms of the things referred to. Micrococci it is convenient to call either by that name or simply spheroids; the words dumb-bell, chain, and colony or zoogloœa, may also I think be advantageously retained as preferable to the terms *Diplobacteria*, *Streptobacteria*, and *Gliabacteria*, which Billroth proposes to substitute for them. In addition to these, the word *mycosis* may be conveniently used to indicate the infiltration of a living tissue with micrococci.

The two diseases of which the names stand at the head of the following sections have been placed together because in each of them the local pathological changes are associated with the development of ordinary micrococci in the affected parts, *i.e.*, with mycosis. Both of them present more or less of the characters of specific contagious diseases; but of neither can it be shown that its specificity (admitting that it is specific) is related to that of the form of vegetation with which the diseased tissues are infested.

Diphtheria.

The term diphtheria, like a great many other names of diseases, is used in two senses. By pathological writers, and especially by surgical pathologists, it is constantly applied to a particular mode of ulceration in which the gradual necrosis of the affected tissue proceeds in such a way as to lead to the formation of a membranous slough.

By nosologists, on the other hand, it is used in its original sense, as the name of a specific contagious sore throat, distinguished from scarlatina by the absence of the eruption, and by its habitual tendency to result in laryngeal croup. It is in the latter sense only that the word can be correctly employed. As, however, the correspondence between the so-called traumatic diphtheritis and the pathological *process* of which the fauces are the seat in contagious sore throat, is more than merely apparent, it is important to take some account of what has been ascertained as to the nature of the surgical affection.

Traumatic affections to which the word diphtheritic is ordinarily applied by surgical writers.

The writers who have principally discussed the relation between surgical diphtheritis and mycosis are Hueter and Billroth. Billroth defines traumatic diphtheritis as fibrinous infiltration,* *i.e.*, a spreading inflammation of the skin chiefly characterised by the induration of the infiltrated part, the stiffness being due to the coagulability of the infiltrating liquid. This he calls Diphtheritis, even when the induration eventually results in the formation of pus or in the gradual restoration of the part to its natural state; in this way he is led to include in his definition cases of erysipelas in which there is much induration or stiffening of the inflamed area. Ulceration or loss of substance is not regarded by Billroth as essential, for although he includes those processes of progressive necrosis beginning from a wound, of which hospital gangrene is the type, under the term diptheritis, he admits their title to be so called, not on the ground of their being ulcerative, but on account of the fibrinous character of the infiltration. As regards all these processes he maintains that the filling of the interstices of the tissue with coagulum always precedes the phagedænic ulceration. Hueter agrees with Billroth in regarding surgical diphtheritis as a non-suppurative acute inflammatory infiltration of the integument spreading from a wound, but would confine the word strictly to processes result-

* Billroth, loc. cit. p. 179.

ing in ulceration or destruction of tissue,* while Billroth would extend its meaning to all acute fibrinous infiltrations, and consequently to many cases of erysipelas.

It is scarcely necessary to state that neither of these definitions identify surgical diphtheria with contagious sore throat; but as regards the relation between the morbid process and the mycosis, the two affections are, as we shall see, remarkably comparable with each other. In both we have to do with a spreading necrosis of a previously infiltrated part, in both the lymphatic system is invaded by micrococci; and the question to be solved is whether the previous infiltration is determined by their presence. As regards surgical diptheritis, we have simply a conflict of observation: both parties admit that the interstitial impletion of the tissue with a granular material is the very first change which occurs. Billroth (grounding his statement, so far as I can make out, on the microscopical examination of a single case of hospital gangrene) holds that the granular material is simply fibrin. Hueter, on the other hand, states that the granules are micrococci, and that the process consists primarily in an invasion of the tissue by these organisms. In support of this he records cases of diphtheritic ulceration in which he investigated the condition of the tissues of the skin at the advancing edge of the infiltration, and consequently at the earliest stage of the process, and found that micrococci could be detected in parts where as yet no structural changes and no other formed elements could be recognised. The question is one of difficulty, not merely because the investigation is of such a nature as to demand the greatest anatomical skill, but because cases affording the required material are fortunately of rare occurrence.

Diphtheria proper, or contagious sore throat.

I now proceed to the consideration of diphtheria as understood by the nosologist. For information as to the etiology, symptoms, and pathology of the disease, I cannot refer to any better source than the papers published in 1860 in the Second Report of the Medical Officer of the Privy Council. The question with which we have to occupy ourselves is that of the nature of the *granular* infiltration of the mucosa and of the tissues immediately subjacent to it, which has been long recognised as the necessary precursor of the loss of substance, both in the fauces and in the larynx. In order to place the reader in a position to judge of this question, I will endeavour to give as clear a summary as I can of what is now known as to the local process.

Summary of the more obvious local changes which have their seat in the mucous membrane of the fauces.

In its beginning the process cannot be distinguished from a common catarrh. A contagious sore throat, like an ordinary angina, takes its start somewhere in that region which from its lying between the nares and larynx, may be regarded as belonging alike to the respiratory and alimentary tract, and consists in redness of the uvula, arches, and tonsils, attended with a greater or less degree of pain or discomfort.

The next change has its seat in the epithelium of the same parts; small greyish white spots appear here and there on the surface of the deeply congested membrane, and are mostly of round or oval contour. This appearance is due to a change in the epithelium to an extent corresponding to the area of each spot or patch. The more superficial layers of that structure are thickened and loosened by being soaked with exudation, to such a degree that the slightest touch is sufficient to detach them. After the detachment of the patch, a surface is left behind which scarcely differs from that which surrounds it, for the deep layer of the epithelium still remains. If the soft white membrane is

* Hueter, Pilzsporen in den Geweben und im Blut bei Gangraena diphtheritica.—Ueber Diphtheritis. Centralblatt f. d. med. Wissenchaften, 1868 pp. 177 & 531

examined microscopically, it is seen that it consists entirely of strata of epithelial scales, which, excepting that they are loosened, and are more or less beset with fine granular matter, present normal characters. So far we have nothing before us which can be said to be distinctive of specific angina, for although it is certainly not usual to see the detachment of the epithelium in continuous patches in other forms of sore throat, yet there is no doubt that it may and does occur to a certain extent frequently, and in so far as this happens there is nothing to distinguish them from diphtheria.

The more important changes which follow, have their seat not in the epithelium, but in the immediately subjacent layers of the mucosa. The peeling of the superficial strata of the epithelium having extended over the whole or a great part of the fauces, and the deep layers being thus denuded, new membranous concretions or sloughs, as they are often called, take the place of those which have been detached. These differ both in appearance and consistence from their predecessors. They possess a brownish or dirty grey colour, and are usually firmer and more resistant. On attempting to remove them, it is found that the purpose can only be accomplished at the expense of the tissue of the mucosa; a raw and bleeding surface is left, which is obviously not epithelial.

Before proceeding to state what has been ascertained as to the nature of some of the finer anatomical changes which give rise to these appearances, it may perhaps be useful to refer very shortly to the normal structure of the mucous membrane of the pharynx in those parts which are the favourite seats of the diphtheritic process. In a vertical section of the mucous membrane of the velum or arches of the palate, it is seen that the deep layer of the epithelium (the *rete Malpighii*) is limited towards the mucosa by a well-defined line, the position of which corresponds to that of the basement membrane of the older anatomists. Underneath this comes a narrow band of apparently homogeneous tissue often called the *membrana propria*. Where the mucosa is papillated, this band limits the outlines of the papillæ; but in the parts in question the papillæ are, at all events in young persons, very little developed. The layer which immediately underlies the propria is seen in section as a very close meshwork of transparent fasciculi, the interspaces of which present various appearances according as they correspond to capillary blood vessels or lymphatic interfascicular channels. Of these the former belong to the extremely abundant vascular network which in injected preparations is seen to lie immediately underneath the *rete Malpighii*, its loops reaching to the very surface of the *membrana propria*, and following all its papillary projections. The interfascicular channels of the mucosa communicate first with the superficial plexus of true lymphatic vessels which lies underneath the fine network of capillaries of the papillary layer, and these with the more deeply placed lymphatics of the sub-mucosa, while in the other direction they lose themselves in the *membrana propria*, which though in ordinary sections apparently structureless, can be shown by appropriate modes of preparation to be permeated by cavities containing living nucleated cells, which communicate with their neighbours in every direction by protoplasmic branches. Continuing our examination downwards, we come lastly to the looser texture of the sub-mucosa, in which, in addition to branches of arteries, veins, and nerves, the mucous glands, when present, are embedded; all these structures being in relation superficially with the mucosa, deeply with the muscles.*

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* See E. Verson, Kehlkopf und Trachea. Stricker's Handbuch der Gewebelehre, vol I. p. 453.

Finer structural changes. Recent investigations of Nassiloff as to the invasion of the tissue of the mucosa, and impletion of its lymphatic channels with masses of micrococci.

The changes which take place in the mucosa have been recently investigated by Nassiloff,* who finds that in sections prepared from mucous membranes in which the diphtheritic process is in progress, but not too advanced, it is seen not merely that the cells of the *rete Malpighii* have undergone remarkable changes, to which it is not needful to refer, but that in the mucosa the interfascicular channels are occupied with similar material, so that they present themselves as a system of opaque granular tracts which intercommunicate with each other, so as to form an imperfect network embedded in a transparent stratum or ground. Proceeding towards the deeper structures, it is seen that the lymphatics with which these channels communicate are filled with similar material, so that in the deeper layer of the mucosa and in the submucosa these vessels present themselves as round, oval, or oblong areas of granulation. This granular material is not, according to Nassiloff, either fibrin or detritus, but consists mainly of micrococci, so that he considers himself justified in regarding the changes which occur in the mucous membrane in faucial diphtheria as probably dependent on the occupation of the channels of absorption by masses of vegetation. As yet nothing has been said of the diphtheritic process as it presents itself in the air passages. In the larynx and trachea its characters differ so strikingly from those met with in the fauces and pharynx, that the most attentive observers, whose means of investigation have been limited to the use of the scalpel, have been led to regard the laryngeal croup as a complication of the faucial angina, not as a continuation of the same process. So far as regards the changes in the epithelium, it would not be difficult to show that the contrast may be attributed to the structural difference between the respiratory and alimentary mucous membranes.

Structural changes which have their seat in the trachea and larynx.

As regards the changes in the mucosa and deeper parts, there appears to be no ground for making any distinction between the mucous membrane of the air passages and that of the fauces. The author already referred to has shown that the micrococcus infiltration can be traced both in the larynx and nares, not merely to the lymphatics of the submucosa, but to the deeper structures—in the former to the cartilaginous tissue, and in the latter to the bony tissue with which it is in relation.

I have already indicated that the micrococci which are found in the diseased tissues in diphtheria cannot be regarded as the generators of the specific contagium (supposing such to exist), the obvious reasons being, first, that the micrococci themselves possess no specific characters, and secondly, that a mycosis of the same kind as that which is associated with diphtheria, occurs in similar connection with other morbid processes which are certainly not specific. There is, nevertheless, another question relating to the subject which still remains open. However certain it may be that they neither embody nor produce any specific contagium, there is still a sense in which they may be pathogenic. The fact that they are present in the earliest stages of the diphtheritic process, and that they find their way even to the extreme limits of the diseased area, would not, even if confirmed by a much larger number of observations than have yet been recorded, be adequate to prove that they are the exciting causes of the subsequent changes. The question is one which can only be answered by experiment, and I now propose to give an account of some efforts which have been made in this direction.

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* Nassiloff, Ueber die Diphtheritis. Virchow's Archiv, vol. L., p. 551. See also on the same subject, Oertel, Experimentelle Untersuchungen über Diphtherie. Deutsches Archiv, vol. VIII., p. 3.

Experimental propagation of the diphtheritic process in animals.

Of the various attempts that have been made to propagate the diphtheritic process in the tissues of healthy animals, almost all have been undertaken in the hope of communicating the disease. I am not indeed certain that any of the experimenters have kept in mind in their investigations the distinction just referred to, between the property of communicating a specific disease, *e. g.* Diphtheria with its sequelæ, in its entirety, and that of merely ingrafting by inoculation, a particular local process.

Some of these experiments have consisted in inserting diphtheritic material in the tissue of the cornea by puncture, others in injecting it subcutaneously,* and others in introducing concretions into the trachea or larynx.† As the most important results have been obtained by the first of these methods, I propose to refer to these exclusively.

There are several reasons why the cornea is preferable to any other tissue for experiments on the propagation of mycosis. Its tissue is better known histologically than any other, and it is closely related in structure to those which are chiefly liable to be the seat of mycoses elsewhere, viz., the superficial layer of the cutis, and the *membrana propria* of the mucous membranes. In addition to this it is the structure in which the phenomena of reaction against injury—those of tissue-germination, and corpuscular infiltration—have been studied with most care.

Artificial production of mycosis of the cornea by inoculation.

The earliest experiments were, I believe, those made by Nassiloff‡ at Professor von Recklinghausen's suggestion in 1869. His method consisted in simply puncturing the cornea of a rabbit with a needle charged with diphtheritic concretion. The injury gave rise to some hyperæmia of the conjunctiva with purulent discharge, which after two or three days subsided, leaving the cornea of a yellowish colour. On microscopic examination this appearance was found to be due to the impletion of the lymphatic canaliculi with micrococci. In one of the experiments this was accompanied by corpuscular infiltration of the peripheral part of the cornea, the white opacity due to this cause being distinguishable even to the naked eye from the brownish yellow tinge which indicated the mycosis. In these experiments the material was derived in one instance from the pharynx of a diphtheritic child, in the other from a case of hospital gangrene. In both, the material appeared to consist entirely of micrococci.

The next experiments were those of Professor Eberth, of Zurich.§ The material was also derived from faucial diphtheritis. The inoculation was performed by making a number of minute punctures in the cornea, and then introducing the fresh diphtheritic concretion into the conjunctival cavity, care being taken to employ instruments that had never before been used. At the same time the opposite cornea was punctured in the same manner without introducing any infecting material, in order to distinguish the merely traumatic results from those due to the contamination.

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* The most important experiments as to the communicability of the diphtheritic process to animals by subcutaneous injection are those of Trendelenburg—Ueber die Contagiosität und locale Natur der Diphtheritis. Archiv f. klin. Chir., vol. x., p. 720.

† The method of introducing concretions into the larynx was employed by Letzerich and Oertel. Both of these experimenters succeeded in producing in rabbits a disease which corresponded with diphtheria as well in its local as in its general manifestations. As, however, the significance of the results obtained by them seems to admit of question, I think it undesirable to refer to them in the text, but have added a note on the subject at the end of this paper.

‡ Nassiloff, loc. cit.

§ Eberth, Zur Kenntniss der bacteritischen Mycosen. Leipzig, 1872, p. 6.

When this method was used it was found that in the uninfected eye the reaction was confined to the appearance of a slight opacity round each prick, which in a couple of days had disappeared. The other in 24 hours was in a state of advanced suppurative inflammation. On examining the cornea with a low power (after the animal had been killed), each puncture presented itself as a greyish brown patch of stellate contour on the anterior surface of the organ. At a deeper level in the corneal tissue, streaks of the same colour were seen stretching from the seat of puncture and intersecting each other in various directions, corresponding to those of the lymphatic canaliculi. On making sections of the organ and examining them with a higher power, the yellow granular substance was found to consist entirely of micrococci.

From these experiments Eberth concludes that the invasion of a tissue by micrococci may result in its disintegration either by the directly destructive action of the organisms themselves, or by the inflammatory reaction to which they give rise, but that in either case they are the primary agents, the efficient causes, by the operation of which the process originates.

Both Eberth and Nassiloff made comparative experiments with ordinary putrid liquids, for the purpose of ascertaining whether results of the same kind as those described above could be thus obtained. These were for the most part negative, showing apparently that micrococci bred or nourished in tissues actually undergoing diphtheritic processes acquired thereby such a habit as fitted them for colonising in a new tissue more readily than others. Subsequently, however, Eberth * published other experiments in which he showed that corneal mycosis could be brought about by any injury of which the mechanical conditions were favourable to contamination from the ordinary sources. Thus, for example, he found that even in corneas inflamed by a thread seton, a certain degree of micrococcus infiltration could be made out in the neighbourhood of the wound. He also found that in the affection of the cornea produced by section of the fifth nerve the disintegrative process was in its origin in great measure mycotic.

As, however, the question still appeared to be doubtful, a new and very extended series of experiments was undertaken by Dr. Dolschenkow in the pathological laboratory of Professor von Recklinghausen, at Strasburg.† The materials used in these experiments were not specific. In some the white or brownish smeary *enduit*, consisting entirely of micrococci and bacteria, which covers putrescent muscle when kept in moist air, was used, in others the scum from putrescent animal liquids. Each inoculation was made by a single puncture in the upper half of the cornea. 112 experiments were made, of which 29 were entirely successful, and 31 partially so. In the remainder the results were negative. The effects were as follows:—After 12 hours a slightly prominent opacity appeared at the seat of each inoculation, the conjunctiva being hyperæmic and slightly œdematous. Six hours later the opacity had increased to the size of a hemp seed; in its centre was a small ulcer, around which the cornea was yellowish, the rest of it beginning to show grey opalescence. At the end of 24 hours the ulcer had increased to the size of a hemp seed and had become deeper, the epithelium of the cornea was detached and the general opalescence had

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* Eberth, Die diphtheritischen Processe. Keratitis nach Trigeminusdurchschneidung. Centralblatt, 1873, pp. 113 and 502.

† Dolschenkow, Impfung faulender Substanzen auf Kaninchenhornhaut; Centralblatt, 1873 p. 655.

passed into opacity. There was already hypopion $\frac{1}{20}''$ deep, and the cavity of the conjunctiva was full of purulent liquid. During the next two days the ulceration of the cornea progressed towards its margin, and eventually, about the end of the third day, perforation usually took place, the conjunctival inflammation having already subsided.

Most of the animals appear to have been killed for microscopical examination about the second day, but there is a want of precise statement upon this point. In each case sections of the prepared cornea made parallel to the ulcerated surface, and at different distances from it, were investigated. Nearest the surface the canaliculi were found to be so occupied with corpuscles and detritus that the extent to which micrococci were present was difficult to determine. In the succeeding sections the corpuscular infiltration was less, and diminished more and more as the deeper strata were reached. Here, although the tissue was perfectly transparent, there were numerous canaliculi which were filled with micrococci to a breadth of $\frac{1}{6000}''$. They could indeed be traced as far as Descemet's membrane, and it was in these deepest layers that the channels showed themselves most beautifully as long slender streaks which exhibited a granular appearance and distinctly brown colour. In addition to the micrococci contained in the canaliculi, isolated microphytes were scattered in the ground substance, most of them following in their distribution the course of the fibrillation. In form the greater number of these were rod-like. The author was unable to discover any micrococci either in the other tissues of the eye, in the pus of the hypopion, or elsewhere in the bodies of the animals.

It was stated above that 46 per cent. of the experiments failed. These failures were attributed by Dr. Dolschenkow in some instances to defective manipulation. Of the remainder a few could only be accounted for as resulting from insusceptibility on the part of the animals used; but the greater number appeared to be dependent on the nature or condition of the infecting material. With reference to this point it was found first, that no result was obtained unless the micrococci were introduced in mass, and secondly, that it was only at an early stage of their development, that is of the putrefactive process, that they possessed a sufficient infective intensity to insure positive results. In comparing these experiments with those of Eberth in which the material employed was of diphtheritic or at all events of pathological origin, it is to be noted that the quantity used by the latter was very much smaller, so that the results, even if they weaken Eberth's inference as to the greater infectiveness of his diphtheritic micrococci, cannot be said to dispose of it altogether. It must be admitted that the subject is still in some obscurity. It is, however, of moment to have learnt that forms of micrococci exist which possess the power of colonizing in living tissues, and thereby inducing a variety of inflammation which is distinguished from others by its tendency to result in disintegration, and that this faculty of originating disintegrative inflammation is possessed by them independently, and can be exercised without the concurrence of any previously existing morbid process.

Erysipelas.

Definition of the term erysipelas.

In the following paragraphs the word erysipelas is understood in its stricter rather than in its wider sense. The most obvious character by which an erysipelas is distinguished from any other inflammation of the skin, as for example that which is present in a burn of sufficient intensity to produce vesication, consists in the fact that it originates from a focus of infection. The way in which it spreads from such a focus over the

surface of the skin may be aptly compared to that in which the process of combustion gradually extends over a sheet of brown paper from any point which has been set fire to. As a rule the infective focus of an erysipelas is a wound, excoriation, or other breach of surface. Sometimes it happens that no traumatic cause can be discovered, but even here it is not the less evident from the form and characters which the patch of redness presents at its first appearance, that the spreading inflammatory process has started either from one centre of origin or from several neighbouring centres at the same time. In either case it is equally plain that a local infection has occurred, *i.e.*, that a contagium has been inserted or otherwise come into existence in the tissue of the affected part.

Summary of the more obvious local phenomena.

Before proceeding to discuss the anatomy of the process of erysipelas it may be well to refer very shortly to some of the obvious changes which the skin undergoes. The red patch with which erysipelas begins, is always limited by a sharply defined contour line, which may be termed the line of extension. Beyond this line, that is further from the centre of origin, the skin is apparently normal; immediately within, it is not only redder than it is over the rest of the affected surface, but turgid from infiltration of interstitial liquid or tissue-juice; this turgidity gives rise to a raised border readily felt by the finger in passing from the healthy to the affected surface. A spreading inflammation of the skin of which the order of extension has the characters just described must be regarded as an erysipelas whether it occurs in man or the lower animals, whether it is induced or of apparently spontaneous origin. It is remarkable that up to a comparatively recent period the finer anatomical changes that occur in the skin in erysipelas were not investigated; the intense redness appeared to be so completely explained by the injection and distension of the blood vessels of the cutis, and the swelling by the soaking of its tissue with serous fluid, that these two changes, viz., congestion and infiltration, were taken as the only elements of the process; for it appeared to be very improbable that an inflammation which does not as a rule affect the same part for more than two or three days, and when it disappears, leaves no visible trace behind it, should be attended with any structural alteration in the tissues.

Structural changes.

The first serious investigation of the histological changes which occur in erysipelas was made six years ago by Volkmann and Steudener,* the material being derived from cases which occurred in the course of an outbreak of the disease at Halle. The principal difficulty in this investigation was, as may be readily supposed, that of obtaining specimens of skin for microscopical examination in which the process was still progressive; for in such cases of erysipelas as result in death, it usually happens that the extension ceases entirely some time before the fatal issue. In three instances, however, out of sixty examined, the process was still extending. The changes described by Volkmann and Steudener as they present themselves in vertical sections of erysipelatous skin in the stage of progress are as follows:—In the superficial layer of the corium the most prominent feature is the vascular injection, which is so intense that the papillæ look as if they consisted entirely of loops of distended capillaries. In addition to this, the vessels are seen to be surrounded by cells in great numbers which present the well-known characters of the colourless corpuscles of the blood; in the deeper layers of the cutis these are so numerous that they form a continuous sheathing to each vessel,

* Volkmann und Steudener, Zur pathologischen Anatomie des Erysipelas. Centralblatt, 1868, p. 561.

these sheaths communicating with each other by tracts of corpuscles, of which the arrangement corresponds to that of the network of interfascicular spaces of the connective tissue.

It was formerly supposed that erysipelas proper could be distinguished from purulent subcutaneous cellulitis not merely by the absence of suppuration, but by the circumstance that in the former disease the effusion or infiltration has its exclusive seat in the cutis. Volkmann and Steudener's investigations show that neither of these distinctions has any sufficient foundation ; for there is no difference between the two diseases, either as regards the characters of the individual migratory corpuscles, or as to their proportionate prevalence in the different layers of the integument. In erysipelas, as in other forms of inflammation of the skin, the chief seat of corpuscular infiltration is not the cutis but the cellular tissue; for whereas in the former, particularly in its superficial layers, the number of migratory corpuscles is relatively very small, the tracts of infiltration that surround the vessels of the subcutaneous cellular tissue, as well as those which follow the course of the connective tissue bundles and surround the groups of fat cells, are often of such a size that a single tract may occupy the whole field of the microscope, and can be readily distinguished by the naked eye in vertical sections as a streak of white opacity. In the subcutaneous adipose tissue the corpuscles are seen not only to surround the masses, but to penetrate between the individual fat cells, following here as elewhere the distribution of the vascular system. As soon as the process begins to recede and the skin regains its natural colour, or assumes the peculiar yellow tinge usually seen after erysipelas, the emigrated cells undergo rapid degeneration, so that after a day or less, all that remains of them is granular detritus. This process goes on so speedily that in the course of three or four days no trace of what has occurred can be detected, the *restitutio ad integrum* being as complete when scrutinised by the aid of the microscope as it appears to be to the naked eye.

Bearing of the preceding facts on the pathology of erysipelas.

The observations of Volkmann and Steudener, although they are of great value as contributions to the anatomy of erysipelas, do not materially aid the comprehension of those attributes of the process from which it derives its right to be regarded as infective ; for the structural changes described by them for the first time, afford no better criterion than we possessed before for distinguishing the erysipelatous from other forms of inflammation ; for if, as Volkmann justly remarks, we were to produce an inflammation of the skin by the application of strong solution of iodine or otherwise, we should find the vascular congestion, the corpuscular infiltration, and the other finer textural changes presenting just the same characters as in erysipelas ; so that if we were to depend upon the aid of the microscope we should find it even more difficult to discriminate between the one process and the other than when we relied on the mere naked eye appearances. In relation to our inquiry, these results are chiefly of value in so far as they enable us to get rid of previously existing anatomical misconceptions. So long as it was supposed that the erysipelatous inflammation had, or at all events might have, a special anatomy of its own, the attention of pathologists was too apt to be drawn from the study of the process itself to structural changes in the tissues affected. Here, as in other instances in which we know what is going on only by its effects, the observer must guard himself against allowing his attention to be so engrossed with the characteristics of a process that the process itself is concealed from him. It is evident that in every erysipelas, changes must have taken place in the tissue at a period antecedent to the corpuscular infiltration, and even to the effusion. The question is, do these early changes leave any

trace behind them? If such traces exist, they will be found not in the inflamed skin but in the skin about to become inflamed; not at the swollen line of extension but beyond it.

Two attempts have lately been made to elucidate this question; one of them by Dr. Orth, of the Pathological Institute in Berlin; the other by Dr. Lukomsky, under the direction of Professor v. Recklinghausen, of Strasburg. Of the first of these researches I propose to give a short abstract; the second is of such importance and value in relation to our inquiry that it seems desirable to reproduce the whole of the author's observations and experiments in a condensed form.

Dr. Orth's experimental researches on erysipelas.

The purpose of Dr. Orth's inquiry* was to ascertain, first, whether it is possible to communicate erysipelas to the lower animals by inoculation of the products of erysipelatous inflammation, and secondly, whether the specific activity of such products depends upon the living organisms (micrococci, bacteria, &c.) they contain. With reference to the first question, a large number of experiments were made, which showed that when the liquid of an erysipelatous bulla or other inflammatory product, is injected subcutaneously in the backs of rabbits, a spreading inflammation is produced which corresponds in every important respect with erysipelas. The redness and swelling extended from the wound over the whole trunk and extremities, the temperature at the same time rising to 106°–107° Fahr. The redness was most intense in the immediate neighbourhood of the line of extension, along which it was limited by a well-defined swollen margin; in the other direction it faded off gradually into the yellow tinge which prevailed generally where the inflammation had already subsided. On dissection, the red parts exhibited marked hyperæmia of the vessels of the skin, along with œdema and loss of transparency of the subcutaneous cellular tissue; in some cases there were small abscesses under the skin, in others none. On microscopical examination the infiltrating liquid of the swollen parts contained an enormous number of bacteria. The anatomical changes in the cutis corresponded with those of erysipelas described by Volkmann and Steudener.

In a second series of researches a cultivation liquid prepared after Cohn's formula, which had been inoculated by the addition of a few drops of the morbid product above referred to, so as to determine the development of bacteria presumed to be identical with those contained in the added liquid, was used for inoculation in the same way as before. The result was very similar to that obtained in the previous experiments.†

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* Orth, Untersuchungen über Erysipel. Archiv. f. experimentelle Pathologie, Vol. I. p. 81.

† It may probably be admitted that in the experiments above related the disease produced was really erysipelas; but clearly they do not prove or render probable the conclusion derived from them by Dr. Orth, that the material used for the inoculation, owed its activity to a specific contagium peculiar to that disease. In my previous investigations relating to secondary inflammation, I had repeatedly occasion to observe that the subcutaneous injection of irritant liquids gave rise to erysipelatous inflammation, even though the most scrupulous care was taken to avoid the contamination of the liquid used with any infective product whatever; and I have no doubt that if Dr. Orth had guarded himself against error by making comparative experiments, either with chemical irritants of sufficient intensity, such as ammonia, or with products derived from other kinds of infective inflammation, he would have obtained the same results. It may be further noticed that the experiments lose much of their value from the intensity of the effects produced. The rapidity with which the *status quo* is restored after the cessation of the erysipelatous process, is as characteristic of it as the rapidity of its invasion, so that in any attempt to imitate it, the dose of the agent employed ought to be as small as possible. In some of Orth's experiments the results rather presented the appearance of purulent œdema of the subcutaneous tissue than of erysipelas proper.

Dr. Lukomsky's researches.

Clinical and pathological observations.

Dr. Lukomsky's paper* consists of two parts: the first is a record of anatomical investigations made partly by Professor von Recklinghausen, partly by the author; the second relates to experiments made for the purpose of testing the identity of induced erysipelas with the natural disease, and obtaining material for conclusions as to the nature of the infective agent.

Case 1.—Sophia G., æt. 60, affected with heart disease, was transferred from a medical to a surgical ward (March 23), on account of erysipelas of the face of two or three days' duration, which had been preceded by rigors. The disease ran a rapid course and terminated fatally on the third day after the patient's admission (March 26). There was no trace of previous injury of the skin.

Pathological appearances:* March 27.—Fat pretty abundant. Bluish reddening of neck and upper part of chest, whence streaks extend to the back. Slight swelling of right side of upper lip and nose. Excoriations and brown crusts on lips. Swelling of eyelids, particularly left. Much incrustation about inner angle of right eye; tissue soft, collapsed; fluid discharge from crusts. Marked chemosis of right eye; mucous secretion in conjunctival cavity. Excoriations under skin; commencing vesiculation on neck, where subcutaneous tissue is soft and infiltrated with pus. The purulent infiltration extends into the intermuscular connective tissue of the neck, but not beneath the pectoral muscles. Tissue of anterior mediastinum infiltrated with reddish but not purulent liquid. Clear brownish fluid in both pleuræ; similar fluid containing fibrinous flocculi in pericardium. [The heart was enlarged, and exhibited the signs of old valvular disease. Lungs normal, with the exception of slight œdema of right lung.] Œdematous infiltration and redness of fauces, base of tongue, and left arytenoepiglottidean fold. Left kidney small; its surface granular, without ecchymoses; a few white streaks and specks are observed in the medullary cones, which, however, are not distinctly purulent. [No other morbid appearance of importance.]†

Underneath the crusts of dried-up vesicles on the neck, the whole of the cutis and subcutaneous tissue is infiltrated with fluid containing cells. . . . The interstices of the cellular tissue fasciculi of the cutis present in section irregular stellate figures, which are most numerous along the course of vessels. In the subcutaneous tissue the cells are in larger aggregations, so as to form purulent foci, which are connected together by tracts of granular deposit consisting of coagulated fibrin. In the skin of the chest the infiltration was slighter, but here some lymphatic vessels were observed which were filled with micrococci, while in other parts lymphatic cavities occurred in which there were collections of the same organisms. At the edge of the affected part, where the infiltration was very slight, several lymph vessels, recognisable by their varicose contours and the jagged form of their processes, were completely plugged with micrococci. These organisms could occasionally be discovered in the lymphatics of the apparently healthy skin, even as much as nine lines beyond the margin of extension. In the subcutaneous tissue of the chest, commencing cellular infiltration could be made out.

In the skin of the head the inflammatory process was obviously in

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* Lukomsky, Untersuchungen über Erysipel. Virchow's Archiv., Vol. LX., 1874, p. 418.

† The pathological observations, all of which are by Prof. von Recklinghausen, are for the most part translated. Where it has been thought necessary to abridge them, the abridged portions have been enclosed in brackets.

recession, the lymph cavities being filled with small round cells, in process of fatty transformation, and free fat granules. The "streaks" in the kidneys were found to be due to corpuscular infiltration; in the infiltrated parts the blood vessels were plugged with micrococci, in the manner first described by v. Recklinghausen in 1871.* Capillaries filled with micrococci also existed in the muscular tissue of the heart, which, however, were not associated with inflammatory changes.

Case 2.—Dorothea H., æt. 43, was admitted on account of a cystosarcoma of the right breast, which was excised on March 6. On the 8th erysipelatous redness appeared at the edge of the wound, which by the 10th had extended to the left nipple, and thence to the back. Intense fever, unconsciousness, with lividity of affected parts, resulted in death on the 13th without further extension of the erysipelas.

Pathological appearances, March 14.—Surface of wound dry. Dry excoriated patches on the skin of the chest. Skin of axillary cavity reddened hæmorrhagically, and excoriated in places, where it is of doughy consistence. On the left side of the chest the epidermis is peeled off, and here and there vesiculated. . . . [No noteworthy appearances presented themselves in the other viscera.]

Anatomical investigation of skin.—Surface of wound covered with a thin layer of broken-up pus corpuscles and granular detritus. . . Purulent infiltration of the subjacent tissues, with dilated blood vessels and extravasations here and there. . . . Edge of wound exhibits a brownish border a line in width, the cut surface of which is remarkably smooth and shiny. In this part it was found that the cellular tissue fasciculi had a glass-like appearance and swelled very little in acetic acid, and that the blood vessels were dilated with blood in which the corpuscles could not be distinguished. The malpighian layer of the epithelium was altered so as to resemble the *stratum corneum*, the processes between the papillæ being transformed into slender parallel streaks, and the papillæ themselves containing vessels in the same condition as those last mentioned. Between this and the inflamed part the transition was rather abrupt, the cells of the malpighian layer of the intermediate part being more distinct than at the very edge of the wound, but still small and granular. Along the line of commencing inflammation there were dilatation of the vessels and abundant corpuscular infiltration, the cells of the rete being swollen, and some of them exhibiting vacuolation, while the papillary blood vessels were so dilated that they occupied almost the whole width of the papillæ. . . .

[The most important changes were observed in the deeper layers of the subcutaneous tissue.] Almost immediately on the surface of the muscles, micrococci existed in vast numbers, forming extensive colonies of the most various forms—round, oval, cylindrical, or stellate. In some places portions of large lymphatic vessels occurred, completely filled with micrococci, the largest colonies being always found in natural cavities, which were also probably lymphatics. In the neighbourhood of the colonies numerous bodies of stellate form, consisting of micrococci, occurred, which were united by their processes so as to constitute a network. The colonies themselves were most numerous in the tracts of connective tissue between fat masses; but the adipose tissue itself was also occasionally obscured by scattered micrococci. Notwithstanding this deposit of micrococci the tissue was free from corpuscular infiltration. [These facts were observed only in the immediate neighbourhood (to a distance of $2\frac{1}{2}''$) of the wound; beyond this the skin was not examined.]

* v. Recklinghausen, Würzburger Verhandlungen, Jan. 10, 1871.

Case 3.—Mathilde L., æt. 15, admitted into hospital for hip-joint disease. Three weeks after the opening of a large abscess, erysipelas commenced (May 18) at the edge of the wound which had not completely united, rapidly spreading over the whole body, with the exception of the head and one forearm. Intense fever, with vomiting and diarrhœa, supervened, terminating fatally June 7, the progress of the erysipelas having ceased two days previously.

Pathological appearances, June 9.—Body emaciated and pale. Skin of lower limbs covered with flakes, under which it is still reddened; some redness of right arm. An incision 9‴ long in upper third of inner aspect of leg. Purulent infiltration of integument of neck; inner half of left clavicle bare of periosteum and bathed in pus. Sterno-clavicular joint opened. Cellular tissue of the anterior mediastinum infiltrated with pus. Both pleuræ contain muddy liquid, with purulent fibrinous deposits. Lungs here and there adherent. Pericardium contains a little muddy liquid; heart small . . . . . ; its internal surface stained. [No important pathological changes in other viscera, excepting that in a few papillæ of the kidneys there were yellowish streaks, and that the retroperitonæal lymphatic glands were much reddened and swollen and surrounded with purulent infiltration, which extended to the pelvic cavity, and thence through the pelvic wall into the left cotyloid cavity.] Luxation upwards of the head of the right femur. Large quantities of pus outside of the pelvis. Carious kyphosis of the thoracic vertebræ, around which there is an enormous cavity filled with thick yellow pus, containing several bony fragments.

Anatomical investigation of diseased skin.—Excessive corpuscular infiltration of the subcutaneous tissue generally; purulent foci under integument of lower limbs; micrococci occur only occasionally and in small groups in the integument of the legs, the appearances referable to their presence being insignificant. Elsewhere in the integument no micrococci could be discovered. In the lungs, liver, and kidneys (in which last they were found in the streaks above referred to) capillary blood vessels obstructed with micrococci presented themselves repeatedly.

Case 4.—Augustin B., æt. 9, an anæmic child of feeble constitution, was admitted June 18, on account of a swelling in the left axilla, consisting of enlarged lymphatic glands, which was excised on the same day. Erysipelas commenced from the edge of the wound June 21, which soon spread over the chest and back. It then (June 23) receded, the general condition becoming worse, the case terminating on the 24th. Before death an erysipelatous redness appeared on the left arm (which had been previously free).

Pathological appearances, June 26.—[Cavity of wound contains much disintegrated tissue infiltrated with pus. Swelling of integument and peeling of the epidermis of left side of chest. Masses of cheesy infiltrated glands on both sides of neck, extending to ears, many of them containing mortar-like substance. Right pleura contains about 10 ounces of reddish muddy liquid with fibrinous masses, both free and covering the pulmonary surface. Left pleura partly adherent, otherwise in a similar condition to that of the right. Tissue of anterior mediastinum dense, and containing enlarged and degenerated glands. Right costal pleura reddened, especially between the ribs. On the left side the intercostal tissue contains several nodules as large as cherries, which also consist of cheesy substance. Pericardium contains greenish liquid with fibrinous masses, free or attached to the serous surfaces; the membrane itself is thickened, reddened, and externally scattered with ecchymoses.

The right lung exhibits lobular patches of grey consolidation of the lower lobe, which, however, for the most part contains air. Other lobes contain old indurations. Left lung œdematous, otherwise healthy. Bronchial glands enlarged and degenerated and surrounded with dense tissue. Mesenteric glands also enlarged and caseous. Considerable alteration of the solitary follicles, but no ulceration of intestine. No other important lesions.]

Anatomical examination of diseased parts.—From the lower edge of the wound to an extent of several inches, the lymphatics of the skin and their branches were found to be choked with micrococci. This was particularly the case in the two layers of lymph capillaries of the corium, which as is well known lie intermediately between the two networks of blood capillaries. [In the author's drawings the form and arrangement of these networks are well seen. In the superficial layer of lymphatics the channels have varicosities, often jagged processes, which are seen to be continuous with colonies of micrococci, which from their form must be regarded as contained in lymphatic canaliculi (Fig. 2). The lymphatics which lie under the deeper network of blood capillaries of the corium (*stratum internum* of Teichmann) do not present the jagged outline of the others; they run parallel for the most part with the connective tissue bundles and elastic fibres (see Figs. 4 and 5). The drawings illustrate the relations of the vessels of both layers with the blood capillaries, showing particularly as regards the more superficial layer how they occupy the interspaces of the capillary network. At a greater distance than two inches from the wound it was found that the micrococci no longer occupied the lymph vessels, but were met with exclusively in blood capillaries; several loops of such capillaries sometimes occurring together entirely plugged with micrococci. Commonly, however, the plugging did not extend beyond a single branch. In other instances colonies presented themselves at points of bifurcation. In this case the corpuscular infiltration of the affected parts of the skin was only considerable in the deeper layers near the course of vessels. In the subcutaneous tissue it was almost entirely wanting. There was no infiltration with micrococci near the upper margin of the wound.]

Case 5.—Therese S., æt. 60, admitted for a large swelling in the neighbourhood of the left parotid, which was evacuated on June 9 by means of Dieulafoy's aspirator. As on the next day it had again filled, it was laid open by a long incision. It was then found to be a cavernous tumour. On the same day a similar tumour of the breast was excised. Three days afterwards the skin began to redden around the edges of the excision wound; by the 17th erysipelas had spread over the chest, and shortly afterwards extended to the back. On the 23rd the erysipelas subsided, but the general condition became worse, and the patient died on the 27th. Shortly before death there was a new extension of erysipelas in the neighbourhood of the seventh vertebra.

Pathological appearances, June 28.—[Much œdema of the legs; moderate distension of the belly. The cavity of the wound near the parotid contained greyish pus, and the parotid itself was in a state of purulent disintegration. The skin in the neighbourhood of the excision wound is reddened and exhibits peeling of the epidermis. The left pleura contains about a pint of greenish liquid, but there is none in the right. Both the pulmonary and pleural costal surfaces are coated with fibrinous deposits, under which the pleura is reddened in some parts; in others it is adherent. At the lower margin of the left lung there is a gangrenous nodule surrounded by lung tissue in the state of commencing purulent infiltration, as well as smaller foci in other parts of

the lower lobe. The right lung also contains metastatic foci, none of which is as large as a cherry. The other pathological appearances were not important.

The microscopical investigation of the affected skin showed that there was only moderate corpuscular infiltration of the cutis, which did not extend at all to the subcutaneous cellular tissue. Fatty degeneration of the infiltrating cells was already beginning. No micrococci could be observed anywhere in the parts first affected ; but in the region in which the erysipelatous process showed itself only a day before death, the lymphatics as well as the canaliculi of the connective tissue were occupied by them, the appearances being the same as in the other cases.]

In four other cases of erysipelas in which the disease was also receding, it was similarly found that there were no micrococci ; the cells with which the tissue was still abundantly infiltrated being in a state of fatty degeneration.

Experimental researches.

The purpose of the experimental part of the investigation was, as has already been stated, to determine in how far the apparently erysipelatous inflammation of the skin, which can be produced artificially in rabbits by the introduction of liquids containing septogenic organisms, corresponds anatomically with erysipelas in man. The experiments which were made at the instance of von Recklinghausen were com menced after the completion of the clinical part of the inquiry. They were of two kinds ; in some the liquids were injected subcutaneously, in others material containing septic bacteria was applied to wounded surfaces.

Subcutaneous injection of liquids containing micrococci.

1. The contents of a cyst evacuated after death, which had been allowed to stand in ice for 24 hours, were employed. The liquid was of a yellowish red colour, and contained a well-formed gelatinous coagulum. It presented, besides numerous blood corpuscles, micrococci and bacteria in abundance, both scattered and in colonies, which were largely made up of necklaces. There was no trace of putrid odour. A cubic centimeter of this liquid was injected into the skin of the right side of the back of a young rabbit which had been carefully shaved, and some of the coagulum was similarly inserted in the opposite side. The animal died 26 hours after the inoculation. Five hours before death it was collapsed, its temperature having diminished from 104° to 97·7° Fahr. ; livid red streaks extended from the seat of injection on the right side to the middle line ; on the left side, where the coagulum had been inserted, the redness had spread over the whole left flank and left hind limb ; at the middle line the two regions of redness had united, and there was doughy œdema of the integument of the belly posteriorly. On dissection the skin was found to be infiltrated with reddish serous liquid containing innumerable bacteria. Sections of skin hardened in alcohol showed intense vascular injection with numerous extravasations ; pus corpuscles occurred sparingly in the skin, abundantly in the subcutaneous liquid. The sections of skin always exhibited the appearance of a "stellate network of micrococcus," that is, a network formed by the confluence of the branches of a number of stellate masses corresponding in arrangement to the lymphatic canaliculi. The author's drawings show lymphatic vessels completely filled with micrococci. In the internal organs, micrococci existed in the muscular tissue of the heart, in the liver, and in the kidneys.

2. After keeping the liquid used in the above experiment for a week, about 12 minims were injected subcutaneously on the left side of the back of a rabbit. Redness began to extend from the wound about eight

hours after the injection, and gradually spread over the whole of the trunk during the following day. The line of extension was dark red, somewhat hæmorrhagic, and slightly prominent. The animal survived 48 hours. Before death the whole of the affected part acquired a yellowish green tinge. The general appearances found on dissection resembled those described in the previous experiment ; the investigation of the skin again showing that the relation between corpuscular infiltration and infiltration with micrococci is the same in the artificial process as in the natural. At the line of extension the cutis was found to be infiltrated both with young cells and micrococci, but two tenths of an inch or less beyond this line, the cells were wanting, the stellate spaces of the lymphatic canalicular system of the cutis being occupied by a network of micrococcus masses of the kind above described, the direct continuity of which with lymphatic vessels also filled with micrococci could be distinctly made out.

3. Two drops of aqueous infusion of muscle of frog were injected subcutaneously in the back of a rabbit. In this case the animal survived eight days—sufficient time to allow of the recession of the inflammation of the skin, which was at first very extensive. The affected parts were of a yellowish colour, the infiltration of the subcutaneous tissue had disappeared, excepting in the foot, where sufficient quantity was obtained for microscopical examination. In this liquid no organisms could be found ; in sections of affected skin hardened in alcohol, cells in advanced fatty degeneration and detritus were met with, but no trace of micrococci.

(Experiments 4, 5, and 6 are omitted as being similar to the above.)

Summary of the results of the first series of experiments.

The results of the first series are summed up by the author as follows : —(1.) The previous observation, that by subcutaneous injection of liquids containing micrococci, intense spreading inflammation of the subcutaneous tissues, in which the cutis participates, is confirmed. (2.) In this process the micrococci, multiply rapidly in the tissue, distributing themselves principally in the lymphatic canaliculi and vessels. (3.) This process can be induced by a fluid containing micrococci even when it shows no signs of putrescence (*e.g.*, by a fluid obtained from the living body, in which there could be no question of septic change unless the very presence of these organisms is to be considered itself proof of putrescence). (4.) Fluid from a dead body, if free from micrococci and bacteria, produced only a local effect which had no tendency to spread. The contents of erysipelatous bullæ, if free from micrococci, produce no result.*

Application of material containing Micrococci to wounded surfaces.

7. A wound $\frac{4}{10}''$ square having been made in the back of a rabbit, the coagulum referred to in Experiment 1 was applied to it. After 24 hours the wound was covered with a brownish depressed crust, and surrounded by a swollen margin; redness extended in all directions from it. By the fourth day the redness had reached the left groin, and spread thence over the left thigh and to the right side of the back.

8. A wound of similar size having been made in the left side of the back of a rabbit, a portion of the frog's muscle used in Experiment 3 was applied to it for eight hours, being secured by plaster. At the time it was removed the wound was surrounded by a patch of redness and swelling which extended nearly 3 inches in length and $1\frac{1}{4}''$ in breadth, exhibiting numerous hæmorrhages. The edges of the wound

* The two last statements are founded on negative experiments, of which I omit the details.

were red and swollen, and its surface of a dirty white appearance. In portions of skin around the margin of the wound which were removed at this time and hardened in alcohol, the canaliculi and lymphatic vessels were already filled with micrococci. The animal died on the fourth day; the redness and swelling had extended to the middle line of the belly, where there was an œdematous tumour as large as a hazel nut, which on puncture yielded a reddish liquid containing numerous bacteria. In most parts of the affected skin the corpuscular infiltration was so considerable that the appearances, so far as related to the micrococci, were less distinct.*

12. A portion of putrid flesh three days old was applied in the same way to a superficial wound on the left side of the back of a rabbit. On removing it eight hours after, a patch of redness and swelling, the border of which was sharply defined, extended $1\frac{1}{2}''$ towards the left side of the belly. It was œdematous at the edge, and on puncture yielded a liquid containing, besides leucocytes, bacteria and micrococci. Portions of skin at the edge of the wound removed for microscopic examination, showed the presence of micrococci in the canaliculi. Next morning the animal was killed. The affected skin was infiltrated and its sectional surface reddish; the subcutaneous connective tissue was œdematous. On the surface of the fascia covering the muscles small hæmorrhages were observed which on the right side near the vertebral column were confluent. Between these places and the affected skin the vessels were strongly injected. On subsequent microscopical examination it was found that the tissue was full of extravasated coloured and colourless blood corpuscles; the latter had assumed very various forms and contained refractive granules in great numbers. In some of the capillaries that still contained blood, bacteria could be distinguished in the serum. In sections of skin from the neighbourhood of the wound, appearances similar to those in Experiment 8 were observed. "At the part where the progress had been most rapid there "was still considerable deposit of fibrin which extended somewhat "beyond the margin of redness. In this fibrin micrococci were embedded; "it was so granular that the micrococci could not be made out until "the preparation had been treated with acetic acid and warmed. They "were found in moderate numbers occupying the canaliculi."

Experiments 13, 14, 15 and 16 are omitted.

Summary of the results of the second series of experiments.

From this second series of experiments the author concludes (1), that septic material containing micrococci, when applied to a wound, produces intense local inflammation which rapidly affects the surrounding skin; the spreading disease thus produced corresponds in all its phenomena with erysipelas. (2) In this process the bacteria and micrococci penetrate into the skin by means of the canaliculi and lymphatics. They are to be found in the greatest numbers at the periphery of the inflamed patch, and particularly in that part of it where the process of inflammation is progressing most rapidly. (3) The erysipelas thus produced tends to spread in certain directions more than in others. When the wound was in the middle of the back or near it, the progress of the inflammation was most rapid towards the belly, but more rapid backwards than forwards. This the author thinks may depend on the direction of the bundles of connective tissue, or that of the lymphatic vessels, or on the influence of gravitation, but he is unable to arrive at a definite conclusion on the subject.

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* Experiments 9, 10, and 11, although related in great detail and yielding facts of interest, are omitted.

App. No. 1.

Part II.

On Pathology of the Infective Processes, by Dr. Burdon Sanderson.

There are four contagious diseases in respect of which the presence in the contagious liquids, of forms of vegetation differing from those met with either after death in the normal tissues or liquids of the body, or during life in the products of primary or secondary inflammation has been established. These are smallpox, sheep-pox, splenic fever, and relapsing fever.

Discovery of the organisms in vaccine lymph.

The first statements as to the existence of organisms in the lymph of cowpox and smallpox were made by Dr. Keber* of Dantzic in 1868, who regarded them as the carriers, if not the generators, of the active virulent principle. From his description it is evident that although it is not quite in harmony with more recent observations, he had before him the objects which have since been described as micrococci by other authors, and he must therefore be regarded as the discoverer of these bodies.

In my first paper on the pathology of contagium, published in 1870,† I gave an account, accompanied by a woodcut, of certain bodies to which I assigned the name of microzymes, the presence of which I found it was usually possible to demonstrate in vaccine liquid. I was not able, however, to assert that their presence was or was not essential to the infective activity of the virus. About the same time Professor Klebs‡ described them in his Handbook of Pathology. Two years afterwards the subject was treated with much more completeness in its morphological aspects by a distinguished botanist, Professor Ferdinand Cohn§ of Breslau. His observations related both to vaccine and variolous lymph, and led to the interesting discovery that the organisms which I had described in the former are also found in the latter, and present characters so entirely similar that it is not possible to draw any distinction between them, either as regards form or development. I reproduce in full the account he gives of them as seen in fresh vaccine lymph. "Such lymph is "always found to be filled with spheroidal corpuscles, which although "they exhibit molecular motion have no motion of their own. They are "extremely small, and in their reaction on light differ little from the "serum in which they are suspended, on which account they are easily "overlooked. They can be made out with perfect distinctness under an "immersion lens of high power if the illumination is favourable, but "without these advantages it is difficult to detect them. Artificial light "is preferable for the purpose to bad daylight. After they have been "once recognised it is possible to detect them with lower powers. It is "difficult to determine the diameter of the spheroids micrometrically. I "estimate it at about three quarters of a micromillimeter ($\frac{1}{33000}''$). In "addition to these, numerous larger more refractive bodies are found in "the lymph, as regards which it is somewhat difficult to say whether they "are fat globules, or result from the further development of the others. "It seems most probable that they are cells, each of which consists of a "dark central part surrounded by a membrane indicated by a clearer ring, "an appearance often seen in stages of division. In perfectly fresh "preparations most of the corpuscles are simple, others being joined "together in pairs in a form resembling the figure 8. After the

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* Keber, Ueber die microscopischen Bestandtheile der Pocken-Lymphe. Virchow's Archiv., Vol. XLII. p. 112.

† Thirteenth Report of the Medical Officer of the Privy Council.

‡ Klebs, Handbuch der Pathologie, Vol. I. p. 40.

§ Cohn, Organismen in der Pockenlymphe. Virchow's Archiv., Vol. LV. p. 229.

"preparation has been kept, the number of these double cells increases, "and soon chains of four begin to be distinguishable. These chains are "usually curved or in zigzag; their attachment one to another is "evidently very slight, as they can readily be displaced. After one or "two hours numerous necklaces present themselves, usually of eight "joints, which are even more readily broken up than the others. To "this displacement the groups are to be attributed; of these some "resemble sarcinæ, but it is to be observed that no true sarcinæ showing "crucial division present themselves. Obviously the very rapid and "uninterrupted multiplication of the spheroids takes place by a process "of repeated transverse division, in which the septa are parallel, so that "the resulting daughter cells are arranged in necklaces like the spores "of a penicillium. In consequence of their very slight attachment to "each other it is only at the beginning that the original grouping, due "to their mode of development, is traceable. After a few hours' obser- "vation they are seen to be all aggregated into irregular colonies or "clumps, each consisting of 16, 32, or more corpuscles. . . . . The mul- "tiplication of cells lasts for several days, the aggregations becoming "larger and larger. If the cover glass is slightly pressed, the groups "separate and the individuals forming it become isolated. . . . . In "capillary glass tubes the multiplication of colonies sometimes lasts a "long time, so that they acquire considerable size and present themselves "as flocculi. By the formation of mucus-like interstitial substance the "corpuscles become intimately united with each other, the mass being "converted into zoogloœa. When this is the case the size of the indi- "vidual cells increases, their contents becoming more refractive, so as to "resemble oil drops. As these agglomerations are apt to be encrusted "with other bodies derived from the lymph, the characters which indi- "cate their origin become indistinct. It often happens that the changes "described are associated with the appearance of acicular crystals "resembling raphides."

Dr. Weigert's investigations as to the existence of micrococci in the lymphatics of the skin in smallpox.

Some months before the appearance of the paper from which the above description is quoted, Dr. C. Weigert* of Breslau published a short communication founded on the microscopical examination of the skin in persons who had died of smallpox, in which he stated that he had found the lymphatic vessels of the cutis plugged with a granular mass which exhibited all the characters of micrococci. These lymphatic vessels were met with for the most part in the tissue of the corium, underneath and around pustules in various stages of advancement. Here they in some cases occurred so constantly, that they could be found in every vertical section of the skin which comprised a pustule, though they were absent in others. In the more superficial layers of the corium, where they were most numerous, they often presented the appearance of a network. They were also found in the foci of corpuscular infiltration (minute abscesses) which in smallpox occur in the corium in the neighbourhood of pustules; here they for the most part accompanied the blood vessels around which the infiltration had taken place. It is of importance to observe that all the cases in which these appearances presented themselves, were of persons who had died early in the disease, not later than the sixth or seventh day. When, as usually happened, death had occurred at a later period, the micrococcus masses could not be distinguished—a fact which Dr. Weigert was disposed to attribute rather to the effect of the very abundant corpuscular infiltration of the tissue in concealing them, than to their disintegration or removal from the tissue.

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* Weigert, Ueber Bacterien in der Pockenhaut. Centralblatt 1871, p. 609.

It is further noteworthy that although many of the cases were markedly hæmorrhagic, the filling of the absorbents with vegetation was not confined to these cases, and indeed presented itself most distinctly where this character was conspicuously absent.

The fact that organisms of a particular form exist in the lymph of smallpox, taken in connexion with the occurrence of similar organisms in the channels of absorption leading from the pustules, suggests the probability of these having to do with the morbid process, but cannot be accepted as an adequate proof that they possess the property of reproducing or propagating the disease. For the establishment of such proof with respect to any contagium, it would have to be shown either that when deprived of its organisms, though otherwise unaltered, it is deprived of its activity, or that when the organisms are introduced alone, they manifest the contagious property of the liquid or tissue from which they were derived. An imperfect approach towards the first of these demonstrations was made in the experimental investigations set forth in my former paper; for it was shown that many contagious liquids lose their activity when they are deprived of the suspended particles in which whatever organisms they contain are included. The second must, with our present means of observation, be regarded as impossible, on the ground that the bodies in question are so minute that there is not the slightest prospect of our being able to separate them in anything like purity. But here again methods of investigation may perhaps be within reach which, although they do not come up to the strict requirements of the problem, approach it. If in any disease capable of propagation by the insertion of a contagium containing recognisable living organisms, however minute, it were in our power to observe the behaviour of these organisms during and after the act of infection, even if it were impossible to separate them from the contagious liquid or substance along with which they were introduced into the body of the infected person or animal, we should be able, by comparing the transformations or other manifestations of vital activity in the lower organism or parasite, with the functional derangements and textural changes in the higher organism resulting from the infection, to ascertain with the utmost precision the nature of the relation, if any, between the two series of phenomena.

Present position of the question as regards the contagium of smallpox.

As regards most infective or contagious diseases, such a method could not be applied, for we have no sufficient knowledge either of the door by which the contagium enters, or of its whereabouts in the organism after its entrance. In the case of smallpox these difficulties do not exist; for even if it is not possible to state precisely how the contagium is distributed from its original place of entrance, it is sufficient to know that it localizes itself in foci, each of which serves as a centre of origin of a morbid process which is as complete in itself as if there were no other. If we could, imitating Dr. Weigert's method, but carrying it out much further than he did, not merely search for our organisms at the sixth day, but at each successive period in the local process, from the first sign of functional disturbance in the *rete Malpighii* at the seat of the future papule, to the maturation of the pustule, and could trace the two developments—that of plant-life on the one hand and that of pathological and structural change on the other—going on side by side, and, as has been already explained, see that both were correlative manifestations of the same action, the object would, as regards smallpox, be accomplished. But here we are met by an insurmountable practical difficulty. No opportunity is ever likely to be afforded us of investigating the pathological anatomy of smallpox in those stages about which it is most important to obtain information.

Happily comparative pathology affords us a means of circumventing this difficulty. In the sheep there is a disease which, as regards its origin, progress, and local manifestations, is the very counterpart of human smallpox. The virus of sheep-pox, as I had the opportunity of observing in 1869, contains organisms similar to those of smallpox and vaccine. The analogy therefore is complete, so complete, indeed, that whatever can be ascertained by observation as to the behaviour of the organism of sheep-pox within the body of the infected animal, or rather of the relation between that behaviour and the pathological structural changes which take place at each focus of infection, may be applied with the greatest confidence to the elucidation of the corresponding processes in the human disease.

These considerations led to Dr. Klein's being requested to undertake the investigation of which the results are embodied in his paper on the pathology of sheep-pox.* It will probably be admitted by most readers that even if the problem has not been completely solved, the investigation has yielded results of the highest pathological value.

Besides smallpox and sheep-pox, two other diseases were mentioned as examples of the association of specific vegetable forms with contagia —relapsing fever and the splenic fever of cattle and of the horse. In the two following sections will be found a statement of what is known with reference to them.

Splenic Fever.

The first infective disease in respect of which it could be asserted with anything like probability that a specific organism was present, either in the blood or tissues, was splenic fever. The peculiar staff-shaped bodies which are apparently always contained in the liquor sanguinis in this disease were discovered in 1855, or perhaps earlier; but their significance is still matter of dispute, there being great difference of opinion, even among those who admit that they are characteristic of the disease, as to the part they play in the morbid process.

Before proceeding to discuss this question, it will probably be useful to give some account of the phenomena of the disease during life and after death.

Phenomena during life.

In the works of systematic writers on veterinary pathology there is considerable obscurity as to the definition of the term splenic fever, or (to use the German and almost as familiar word) *Milzbrand*. This arises not so much from want of clearness in the language employed, as from the effort to unite a number of clinical forms under one nosological species, without perhaps always taking sufficient pains to distinguish between what is essential and what is accidental.

The most striking feature of the disease is its extremely rapid progress. In the most rapid cases, those to which all writers refer as "apoplectic" (although the term is far from being expressive of what is observed), the disease runs its course in a few hours at most. In the ordinary cases as they occur among cattle, it lasts a day or two, its course being more or less as follows :—

An animal which perhaps for the previous day has declined food and shown signs of general disturbance, begins to shudder and to have twitches of the muscles of the back, and soon after becomes weak and listless. In the meantime the respiration becomes frequent and often difficult, and the temperature rises (Bollinger) to 3 or 4 degrees above the normal; but soon convulsions, affecting chiefly the muscles of the back and loins, usher in the fatal collapse, of which the progress

* Dr. Klein's paper will be found at p. 49.

is marked by complete loss of power of moving the trunk or extremities, diminution of temperature, mucous and sanguinolent alvine evacuations, and similar discharges from the mouth and nares. The visible mucous membrane, congested from the first, becomes intensely so as the disease progresses.

In the horse the disease presents similar characters, but is much less acute. Partly in consequence of this, and partly as a result of the greater attention paid to the more valuable animal by veterinary practitioners, the local affection is more frequently recognised during life, so that the case is often recorded as of "acute splenic tumour." *

Pathological anatomy.

Of the phenomena revealed by dissection, those which relate to the blood itself, to the circulatory and lymphatic systems, and to the spleen (which may be regarded as a part of both), are by far the most important. In the blood the relative number of the colourless corpuscles in all excepting the most rapid cases is very considerably increased. In the horse, in consequence of the usually longer duration of the disease, this increase is so great that the blood in some cases assumes the leucæmic character. The absence of coagulability and the general tendency, not only of the liquor sanguinis, but also of the corpuscles, to soak or squeeze through from blood vessels into all the cavities of the lymphatic system, whether serous or interstitial, shows itself in the former, and particularly in the peritoneum, by the accumulation of large quantities of sanguinolent liquid, by the intense congestion of the membrane, and by the innumerable hæmorrhagic patches with which the congested parts are everywhere beset. In the subcutaneous, submucous, and subserous cellular tissue the same changes are in progress, though not in all directions in the same degree. Under the skin, infiltration of this kind often gives rise to localised swellings, which are apt to be called carbuncles. In the neck it surrounds the pharynx and larynx, and extends downwards by continuity of tissue to the anterior mediastinum, as well as among the muscles. In the abdomen it occupies the retro-peritonæal space, in which all the organs are similarly soaked with liquid, turbid from the presence of corpuscular elements and discoloured by altered blood. To this condition veterinary pathologists have assigned the term typhous infiltration (*gelbe sulzige Infiltration*).

Of the enlargement of the spleen nothing can be said, excepting that it is always observed, and that taken in connection with the "infiltration" of the connective tissue already described, it constitutes the most constant pathological feature of the disease. The enlarged spleen when examined in acute cases is soft and soaked with blood, its capsule often appearing to be distended. In more protracted cases it acquires greater firmness, but does not at first diminish in size. Nothing is known, so far as I am aware, of the structural changes which take place in the splenic tissue, except in so far that even in the acute forms there is reason for believing that the augmentation of volume is not entirely the result of hyperæmia, but is also due to the multiplication of the elements of which the organ is composed.

Finally, in the digestive canal generally, and particularly in the fourth stomach and duodenum, there is usually extreme congestion of the surface and sero-sanguinolent infiltration of the mucosa. In bovine animals this condition extends along the whole course of the intestine, so that it is common to find its whole cavity filled with dark blood-

* Bollinger, Zur Pathologie des Milzbrandes. Munich, 1872. The above description is chiefly founded on the clinical observations contained in this work. The symptomatology of the disease is also fully given in Prof. Korángi's article in the Handbuch der Allgem. und speciellen Chirurgie, Vol. I., part ii., p. 149.

stained liquid. But in the horse the process is localised in particular places. A favourite seat of these so-called typhous or carbuncular infiltrations of the mucous membrane is the pyloric end of the stomach and the adjoining part of the duodenum; here foci of infiltration may be recognised, even before the viscus is opened, by the patches of dark lividity of the serous membrane, and by the enormous thickening of parts of the wall of the organ which correspond to them. If they are cut into when of recent origin, the intensely congested infiltrated masses are found to be beset with extravasations; eventually the central part of each focus is converted into a slough, which on separating, leaves behind it a slowly healing ulcer, often termed "typhous" ulcer. The so-called carbuncles which occur in splenic fever in the horse owe their origin to a similar process. That is, they are to be regarded not as "localizations" of the disease, but as parts of the skin of which the condition differs from the rest merely in the degree of intensity. They are often followed by sloughing of the subcutaneous tissue, with which the general infiltration manifests itself. Among cattle "carbuncles" are rarely met with, so that although one of the names by which the disease is most frequently designated is derived from them, they are by no means characteristic of it.*

Discovery of the organisms of the blood of splenic fever.

The discovery that in the blood of animals affected with splenic fever rodlike bodies are found in the liquor sanguinis, was first, I believe, made by Pollender,† who in the year 1855 described them as "fine, "apparently solid, straight, unbranched objects from $\frac{1}{400}'''$ to $\frac{1}{200}'''$ "in length and $\frac{1}{3000}'''$ in width." He noted that they possessed no proper motion, differing in this respect from ordinary bacteria. The question of their nature as well as that of their relation to the contagion of the disease was left open.

A year or two after the publication of Pollender's paper, Brauell ‡ published the first of his very extensive series of researches on the contagion of splenic fever, in which he not only confirmed Pollender's discovery, but by experiments in which he communicated the disease to animals under a great variety of conditions, acquired for pathologists the greater part of the accurate knowledge they now possess on the subject. He contented himself, however, with demonstrating that the organisms found in the blood in splenic fever were characteristic of the disease, without maintaining that they were or that they were not the *causes* of its virulence, and indeed was disposed to attribute more importance to them as diagnostics than as agents in the morbid process.

Three years later the bodies in question were re-discovered by Delafond and Davaine, who in claiming a priority which did not belong to them, no doubt did so in complete ignorance of the good work that had already been done so well in Germany.

The characteristics of the rods have been recently given by Bollinger on the basis of observations made expressly for the purpose as follows :— The splenic fever bacterium measures usually from 7 to 12 micromilli-

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* The best account of the pathological anatomy of splenic fever is to be found in Brückmüller's Lehrbuch der pathol. Zootomie, Vienna, 1869, p. 260 et passim. The details are founded entirely on original observations.

† Pollender, Microscopische und microchemische Untersuchungen des Milzbrandblutes. Caspar's Vierteljahrschr. f. gerichtliche und öffentliche Medicin, Vol. XIII. p. 103.

‡ Brauell, Versuche und Untersuchungen betreffend den Milzbrand, Virchow's Archiv., 1857, Vol. XI., p. 132. Weitere Mittheilungen über Milzbrand. Virchow Vol. XIV. p. 432.

meters ($= \frac{1}{3500}''$ to $\frac{1}{2000}''$) in length, so that it is about as long as the width of a coloured blood corpuscle. Its width scarcely admits of measurement.*

Along with the rods separate cocci are always to be found in the liquor sanguinis, which are regarded by Bollinger as certainly in organic continuity with them. The rods are entirely destitute of proper movement. In the fresh state they appear to be homogeneous, but after they have been acted on by water, or are beginning to be altered by the commencement of putrefactive change, it is seen that each rod is made up of spheroids, each spheroid consisting of a central darker part with a more transparent envelope. The most remarkable fact relating to them is that they disintegrate rapidly from the moment that the blood becomes putrescent. As they disappear, the actively moving rods and spheroids which indicate the commencement of the septic process take their place, but there is no continuity of development between the two kinds of organisms.

Conditions of communicability of splenic fever

The following are the most important facts relating to the contagion of splenic fever which have been ascertained, either as the result of clinical or experimental inquiry.

1. The disease may be communicated by any means which involves the transference of a portion of the blood of a diseased animal to the living tissue of an animal previously healthy. It is not known that it can be propagated by any other way, for animals kept in the closest proximity to diseased ones, and placed under the most favourable conditions for infection through the air, are not infected.

2. Although the blood of animals affected with splenic fever always contains the staff-shaped bodies if it is examined at a sufficiently advanced period, the disease can be communicated by the inoculation of blood in which these bodies are either not present, or at all events not in such numbers as to admit of their being made out microscopically.

This was originally stated by Brauell, and has been recently confirmed by Bollinger as the result of experiments made expressly for the purpose of arriving at a definite conclusion on the subject. He found that the staff-shaped bodies were just as numerous in the animals infected by the injection of blood which was free from organisms as in others. In order to use this fact as a means of judging in how far the staff-shaped bodies take part in communicating to the blood its contagious property, it is necessary to bring to bear upon it what we know as to the relation of other organisms of the same class to the liquids in which they are produced. It has been proved experimentally that two liquids of the same chemical constitution, and placed under exactly the same conditions (*i.e.* both completely protected from external contamination), may stand in an entirely different relation to the ordinary bacteria which pervade all aqueous media, the difference consisting in this, that whereas one of the liquids is proved to be prone to the breeding of bacteria by their appearing in it as if spontaneously, the other may be kept for any length

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* The existence of the rods as a characteristic of the circulating blood in animals affected with splenic fever is a fact now admitted by all pathologists who are conversant with the disease. It is, however, remarkable (and at first sight difficult to undestand) that many observers have expressed doubts as to their being organised living beings. Thus Leisering (Bericht über das Veterinärwesen im Königr. Sachsen, 1862, p. 29, reported in Canstatt's Jahresb. for 1863), at first took them for fragments of fibrin or for bits of tissue (*Gewebestrümmer*). F. Müller (Physiol. der Haussäugethiere, Wien, 1862, p. 163), thought that they were blood crystals. The statement made by Bruckmüller, and repeated by others (l. c. p. 261) that Virchow regarded the bacteria of splenic fever as blood-crystals, appears to be an erroneous one founded on a misquotation of an oral communication.

of time without such development taking place. The difference between the two liquids (both of which are, of course, supposed to be placed under circumstances favourable to the growth of bacteria), or rather between the properties which they severally manifest, is usually designated by the terms sterile and fruitful, of which the latter denotes that the liquid to which it is applied contains something which although inappreciable by the microscope, is already in organic development.

As regards the rods of splenic fever, it is not only possible but much more probable than the contrary, that the case is similar. Without attempting to arrive at a conclusion as to the form in which the organisms conceal themselves, it is sufficient to know that the fact of their absence in liquids of proved virulence is in itself no ground for refusing to admit either that they themselves are the contagium, or are concerned in its production, provided that other reasons exist for believing that it is so. It has been sought to demonstrate this in two ways—on the one hand, by separating the rods from the liquid and determining their pathogenic properties by experiment, and on the other, by testing the activity of the liquid after the rods have been removed from it by some process of filtration. The first of these methods, that employed or rather attempted by Davaine and the French experimenters, has led to no result; and it is difficult to see how it could yield any, first, because complete separation of such organisms from the medium in which they are contained is impossible, and secondly, because in the very act of filtration the organisms themselves would probably undergo such changes as would entirely alter their properties. If, as is probable, the activity of the rods is dependent on their vital functions, there is no reason to anticipate that they would continue to manifest that activity when "out of their element," still less that it would show itself in any descendants or progeny obtained from them by cultivation. The other method has been used by Klebs and Tiegel.* These observers have found that just as it is possible to separate the bacteria contained in ordinary septic products from the liquid in which they are suspended by filtering them under pressure through a stratum of porous porcelain, the same method can also be successfully applied to blood containing the rods of splenic fever. The result of the experiments, which it must be admitted were not so frequently repeated as could have been wished, was that the filtrate of blood (or rather the liquid of the spleen of a diseased animal), of the virulence of which experimental proof had previously been obtained, was found to be incapable of communicating the disease. Clearly there is no proof in this fact that the contagium of splenic fever is exclusively contained in the rods; but there is proof in it that the agent is, as we believe all other contagia are, a body which is incapable of solution or diffusion, and which attaches itself to concrete *particles*. An additional and to my mind a more satisfactory proof of this lies in the remarkable observation made by Brauell, that when pregnant animals are affected with splenic fever the blood of the embryo is not contagious. Brauell found in repeated trials, that in such animals, blood from the fœtal circulation could be inoculated without results. Here the placental apparatus serves not merely as a filter but as a diffusion cell, keeping back not merely solid particles, but everything which is not capable of passing through animal membranes. Splenic fever is so incapable of being communicated, otherwise than by actual translation of blood, that the fœtus is not infected by the mother.

There are facts relating to splenic fever which show on the one hand

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* Tiegel, Die Ursache des Milzbrandes. Arbeiten aus dem Berner patholog. Institut, herausgegeben von Prof. E. Klebs, 1873, p. 136.

that the contagious property, as it exists in the circulating blood, is very transient, and on the other that there must be a form or state of the contagium in which it is remarkably persistent. The first of these is proved by Professor Brauell, who having discovered, as has already been mentioned, that the blood lost its activity from the moment that it began to show signs of putrescence, made comparative experiments for the purpose of determining at what period after death this took place, and found that after 3–5 days it had lost its activity. He also observed that as soon as the blood began to change, the motionless rods became (as he supposed) transformed into actively moving bacteria. On this point, almost the only one in respect of which his observations appear to have been inexact, he has been corrected by later observers. As has been already stated, there is no organic continuity between the two successive developments. The ubiquitous septic bacteria cannot be regarded as the progeny of their pathogenic predecessors.

Occasional latency of the contagium of splenic fever in certain localities.

That the contagium must have a state of existence in which it is in the highest degree resistant is obvious from what is known as to the distribution of the disease among cattle. On this subject observations were made and collected by Heusinger * more than 20 years ago, showing how the contagium may take up its residence in very limited localities, betraying its presence only at long intervals by the occurrence of a fresh case. A very remarkable series of observations has been published by Bollinger in his work already so often quoted. The author relates the history of what may be called a stable enzootic of splenic disease, which lasted from August 1868 to February 1872. During this period more than 10 cases occurred at intervals at one stable, situated in the middle of the village of Werikon, not far from Zürich; no cases presented themselves anywhere else in the village or neighbourhood, although the stable in question was (as usual in Swiss villages) closely surrounded by others. In whatever form, the contagious agent must have remained latent in the stable during the whole period.

The general fact of which this particular instance is the representative, has long appeared difficult to explain. How does it happen that an acute contagious disease of which the only known mode of transmission is by inoculation, and of which the duration is so ephemeral, does not perish out of existence? Clearly it inevitably would do so, had it not some other as yet unknown means of perpetuating itself. What this means is it would be too bold to conjecture. I have elsewhere given reasons for thinking that the faculty which various contagia possess of maintaining their activity outside of the body, is more easily explained as a function of life and organisation resident in the contagium itself than any other way.

One of the strongest reasons which can be adduced for thinking that in the present instance the rods are concerned in the morbid process, is to be found in the fact that like the contagious property they may be supposed to represent, they are very fugitive, and in particular, that like it and along with it they disappear at the moment that putrefaction commences. It is therefore not possible to refer to them as contrivances suited for the storage of contagium, or for its conveyance to a distance, for either of which purposes they are obviously unfit. This very consideration, however, can I think be made to appear rather as an aid to our understanding of the relation between organisation, or rather living organic form, and contagion than as a difficulty or objection. For if we turn from the specific form to what we know about the development

* Heusinger. Die Milzbrandkrankheiten der Thiere und des Menschen. Erlangen 1850, p. 345.

and life history of common bacteria, and indeed of the beings belonging to the same group in general, we find the same thing. Just as in the case of the specific contagium, we are compelled to recognise two states of existence, one enduring and latent, the other active but fugitive ; so among these plants we find one condition in which the growth and multiplication of individuals and the individual life is extremely short, another in which the vital activities of the organism are stored up for the future, the individual being for this very end endowed with the power of resisting external disintegrating agencies, and thereby of enduring for an indefinite period.

The common ubiquitous bacteria, those which are concerned in putrefactive changes, are known to be in their ordinary active state easily destroyed. Thus they are unable to survive complete desiccation, or a temperature higher than 80° C. On the other hand, it is equally well ascertained that masses containing bacteria are not deprived of the power of originating new generations of these organisms by heat, unless they are either subjected to a temperature considerably higher than that of ebullition, or boiled for a very long period. The reason of the apparent discrepancy is to be found in the fact that the bacteria have two modes of existence, the one characterized by permanence and resistance, the other by rapid development and short duration ; that in all bacteria masses which have the power of resisting high temperature, *e.g.*, in cheese, there exist, in addition to the ordinary forms of readily killed bacteria, other living particles of more stable structure.

The properties of such bodies, to which Professor Cohn assigns the name of lasting spores (*Dauersporen*), are only just now beginning to occupy the attention of mycologists. I have referred to them here in order to show how completely the biography of a schizomyces represents all the phases of the process of contagion.

Acute diseases of man apparently identical with splenic fever—*Mycosis intestinalis.*

During the last few years the pathology of splenic fever has acquired an even wider interest than it had before, from the publication of a series of cases of a rapidly fatal disease affecting the human subject, which presents the characters of splenic fever, and turns out on investigation to be identical with it. The first of these cases was published by Professor Buhl * at Munich in 1868. It is that of a man aged 32, who died after a very short illness, of which the chief feature was rapid collapse with vomiting. After death the stomach and duodenum exhibited a number of circumscribed foci of infiltration with intense congestion, and extravasations of blood in the infiltrated tissue. The spleen and the lymphatic glands in relation with the affected parts were enlarged, and the peritonæum was full of liquid. The ætiology of the disease, *i.e.*, the origin of the infection, was not recognised, but it was found that the foci of infiltration were infested by masses of organisms, which according to Bollinger, who was present at the examination, exhibited the character of the rods of splenic fever. The same structures were found in great abundance in the blood of the portal system. In other parts of the circulation the rods were absent, but spheroidal corpuscles occurred which were described as *conidia.*

The affection was termed by Buhl "*mycosis intestinalis.*" Shortly afterwards two other cases were recorded by Professor Waldeyer, now of Strasburg.† In one of them, that of a slaughterer, whose occupation pointed to the source of infection, the nature of the disease was suspected during life. After death the suspicion was confirmed by the complete

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* Buhl, Mycosis intestinalis. Centralblatt, 1868, p. 3 ; and Zeitschr. f. Biologie, 1870, p. 129. Bollinger, loc. cit. p. 114.

† Waldeyer, Ueber Mycosis intestinalis. Virchow's Archiv. Vol. LII. p. 541.

correspondence of the lesions with those of splenic fever in the horse. As in Buhl's case the microscopical investigation showed that masses of zoogloea existed in the affected tissues, and that rod-like microzymes were to be found in the circulating blood, particularly in that of the portal vein. Other cases soon followed, all rapidly fatal, and all exhibiting pathological appearances of the same kind, the most constant being the enlargement of the spleen, and the circumscribed hæmorrhagic infiltrations of the intestines, resulting in more or less extensive sloughing of the mucous membrane, and associated with serous exudations, infiltration of the subserous tissue, and hyperæmic enlargement of the lymphatic glands in relation with the infiltrated parts.

Whatever doubt remained as to the identity of *mycosis intestinalis* with splenic fever was removed by the appearance of a communication by Dr. Münch of Moscow,* who was able to state from the results of his own dissections in the "Workman's Hospital" at that place, that in all the cases in which he had occasion to examine the bodies of men who had died of disease contracted by infection from animals, the intestinal lesions and the condition of the other internal viscera corresponded closely with those described by Buhl and Waldeyer. In commenting on this paper, Bollinger points out that in Heusinger's great work on splenic fever, a whole series of cases of communication of the disease to man are recorded, in which the appearances after death corresponded with those which have been described, and that in these, as in those recently recorded by Münch, the primary pustule (*pustula maligna*) at the seat of inoculation, often supposed to be the one pathognomonic lesion of splenic fever in man, was by no means a constant feature. Out of 28 cases recorded by Münch (1867–71) seventeen exhibited a primary pustule, which in all but two was carbunculous. But in the other 11 cases, there was nothing whatever to be found on the skin.

As regards the morphological relations of the organisms found in mycosis intestinalis with those of splenic fever, further investigation is required. So far as the former have been described they agree completely with the others, as may be readily seen by comparing the drawings of Wagner,† taken from a case of mycosis, with Cohn's or Bollinger's representations of the "rods" of splenic fever.

The identity of the two forms being admitted, Buhl's and Waldeyer's anatomical investigations afford a key which was before entirely wanting, to the understanding of the so-called "localizations" of splenic fever. This key consists in the demonstration that the "*sulzige Infiltration*," of which we read so constantly and repeatedly in the descriptions of the veterinary pathologists, is a process of which it is *the* characteristic that the tissues of the infiltrated part become infested by myriads of microzymes of a particular kind, so that the presence of these bodies is as much a part of the process as the emigration of the colourless corpuscles from the blood vessels is a part of ordinary suppuration.

Further than this it is not possible to go, until the initial stages of the process, the early structural changes, have been investigated in the lower animals. For this investigation the field is open, and it is in the highest degree desirable, in the interests of humanity, that it should be undertaken. The material is abundant; what is required is that the highest pathological knowledge and anatomical skill should be employed

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* Münch, Mycosis intestinalis und Milzbrand. Centralblatt, 1871, p. 803. Heusinger, loc. cit., p. 597.

† Wagner, Ein Fall von tödtlichen Pilzkrankheit—Mycosis intestiralis. Leipzig, 1872.

in its investigation. If this is done results of the greatest value may be confidently anticipated. We may hope thereby to acquire a knowledge of one more of those malignant processes by which acute disease destroys human as well as animal life, processes of which we may be perfectly certain that their danger is in proportion to our ignorance of their nature.

Relapsing Fever.

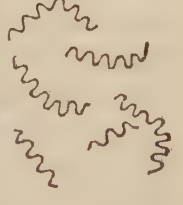
In the epidemic of relapsing fever which prevailed in Berlin from November 1867 to May 1868, Dr. Obermeier, whose attention had been called to the question of the relation between specific organic forms and contagia by the publications of Professor Hallier, investigated the blood microscopically in 82 cases. In general the results were negative. In some cases, however, he noted appearances of which he did not at the time understand the full significance, but was prevented from investigating the subject further by the cessation of the epidemic.

Discovery of the spirilla of relapsing fever.

When relapsing fever again appeared in Berlin in 1872, the inquiry was resumed. In the very first case which was investigated, the bodies, to be referred to in the following paragraphs as *spirilla*, were discovered in the blood. His first communication on the subject to the Berlin Medical Society* was based on 12 cases, in all of which the organisms were observed. On the 26th of March Dr. Obermeier published a second series of observations;† 20 new cases had been investigated, in nine of which the blood had been examined daily. The results of these examinations showed that the presence of the organisms in the blood was associated with the morbid process so closely, that as soon as the pyrexia disappeared they disappeared with it, reappearing when the patient again fevered in the relapse.

Soon after the death of Dr. Obermeier in August 1873,‡ a third series of observations were communicated by Dr. Engel to the Berlin Medical Weekly Journal.§ They were founded on the examination of the blood in 18 cases in the Charité Hospital at Berlin, under the care of Professor Frerichs. They confirmed in every particular the statements of Obermeier, deriving additional value from the exact observations they contained as to the time which intervenes between the onset of pyrexia and the first appearance of the organisms.

During the same year the epidemic prevalence of relapsing fever in Breslau afforded additional opportunities for investigating the subject. The first results of these inquiries were communicated by Dr. Weigert to the medical section of the Silesian Scientific Society on September 12, 1873.‖ The morphological and botanical characters of the organisms were subsequently determined by Professor Cohn, who was kind enough to send me the sketch of which the woodcut is a copy. The clinical relations of the subject have been since worked out by Dr. M. Litten in a report on the epidemic, founded on observations in the Allerheiligen Hospital in Breslau. Of this report I subjoin an abstract.¶



* Report of the meeting of Feb. 26, 1873. Berlin. klin. Wochenschrift, 1873, p. 152.

† l. c., p. 391.

‡ Dr. Obermeier died of cholera while engaged in his scientific investigations.

§ Engel, Ueber die Obermeierschen Recurrens spirillen, l. c., p. 409.

‖ Berlin. klin. Wochenschrift, 1873, p. 589.

¶ Die Recurrens-Epidemie in Breslau im Jahre 1872–73, von Dr. M. Litten Assistenzarzt am Hospital zu Allerheiligen

Clinical observations.

The total number of cases observed by Dr. Litten was 448. Of 400 which were carefully recorded, 43½ per cent. had two paroxysms, 35½ had three paroxysms; in six out of the 400 cases (1½ per cent.) there was only one attack, in seven cases there were four, and in three five attacks. This leaves 17 per cent. of the cases unaccounted for, in which the course of the disease was insufficiently determined.

The mean duration of each paroxysm and intervening period of pyrexia is expressed in the following numbers. The mean duration of the first attack was 6·6 days, of the second 4·9 days, of the third 3·1 days, of the fourth 3·1 days, and of the fifth 2·3 days. The mean duration of the period between the first and second attack was 8 days, that between the second and third 9 days, between the third and fourth 6·3 days, between the fourth and fifth 8·3 days.

Nosological character.

During the paroxysms, the pyrexia for the most part exhibited an intermittent type, in which the morning and evening temperatures differed from each other so much, that the case resembled one of quotidian ague. In many of the cases the temperature rose a degree or two before the critical fall, the rise being accompanied by a shivering of considerable duration, followed by profuse sweating. In the majority, however, the temperature began to sink before the sweating came on. In addition to the ordinarily observed critical phenomena (sweating, epistaxis, vomiting, diarrhœa, deposit of sediment in the urine, &c.), the crisis was accompanied in seven cases by a herpetic eruption about the lips and nose.

The course of the disease as regarded variations of temperature corresponded exactly with those usually observed in relapsing fever. There were, however, some cases in which irregular paroxysms presented themselves. This happened particularly among those patients in which the intensity and duration of the fever gave ground for alarm, although there were no complications. In addition to these there were other cases in which the disease beginning as relapsing fever assumed eventually a distinctly tertian or quotidian form (p. 288), the patients having been previously exposed to malaria; and here it was most interesting to observe that the spirilla, which had been constantly found during the true relapses, were not present during the ague paroxysms. The author records temperature curves of several cases illustrative of these facts.

Of the pathological signs of the disease during life, the enlargement of the spleen was the most constant. It was present in 96 per cent. of the cases, and could be detected much earlier in the disease than is possible in typhus or intermittent. The diminution of the splenic tumour did not take place until some days after each crisis, but the increase began from the very commencement of each relapse. The swelling was always attended with pain in the left hypochondrium; the pain was increased in inspiration. In some cases the enlargement of the spleen was accompanied by a corresponding enlargement of the liver, the duration of which was similar.

The most frequent complication in the epidemic was bronchial catarrh. It occurred mostly at an early period in the disease, often as soon as the second or third day, whereas in typhoid it usually does not happen till the beginning of the second week. In some cases it was attended with œdema of the lungs, which in two weakly old persons terminated fatally. True croupous pneumonia occurred in 28 cases, of which 17 were one-sided, 11 double. The access of the complication was marked in the younger patients by rigors. In all of these cases the sputa were characteristic, and the physical signs were those of genuine lobar pneumonia. Of the 28 cases, of which 23 were fatal, the attack hap-

pened in 11 during the second interval of apyrexia, and in 8 during the first.

Most of the patients suffered during the paroxysms from headache and giddiness; some were somnolent and unable to answer questions; others talkative, but answered rationally when spoken to. In some cases with very high temperatures (42° C.) there were no marked symptoms of cerebral disturbance.

Pains in the joints were a frequent complication, and often lasted for some weeks after the decline of the disease. Usually, however, they were experienced only during the paroxysms. Still more constantly the patients suffered from severe pains in the muscles, affecting either groups of muscles or the whole muscular system. The author dwells on the painful and distressing character of this symptom as experienced by himself. It usually declined during the apyrexia, recurring with greater intensity in the relapse. The muscles usually affected were those of the neck and lower extremities. The spirilla could not be detected in the muscular tissue.

Of the 448 cases observed, 32 died (7·2 per cent.), five in the first apyrexia, eight in the second paroxysm, 15 in the second apyrexia, and the remaining four in subsequent stages. With reference to the direct causes of death, 23 were attributed to pneumonia, as above stated; five persons died without complications at the height of the fever, no direct cause of death being made out at the autopsy.

Origin and progress of the epidemic.

Dr. Litten begins his history of the epidemic with a very complete account of the earliest cases, and of their relation with each other. The first person attacked was a female tramp, who came to Breslau from another district (Neisse), in which no previous case was known to have occurred, lodged for the night in a Refuge, and took ill on the following day. The second case was that of a woman who had slept in the next bed to the other. Both patients in succession were admitted into the Allerheiligen Hospital, soon after which a woman convalescent from erysipelas, who occupied the next bed to one of the patients, and a nurse were attacked. A married sister of the nurse and three of her children subsequently became ill. In the meantime a separate focus had established itself out of the town, in a hamlet called Wilhelmsruh. Here 14 persons, belonging to four families, *all of which inhabited the same room*, were attacked; all the other inhabitants of the hamlet, although some of them occupied rooms in the same building, remained free. They were transferred to a previously unoccupied building which had just been opened as a Refuge in the town. Into this three other persons not affected with the disease were also received, of whom one only escaped infection. Several persons who left the Refuge became the centres of "tenement-epidemics" (*Stuben Epidemien*), in which as a rule nearly all the inmates of the invaded houses were attacked; but the disease did not spread in localities from which the affected persons were removed.

During the progress of the epidemic 21 persons, chiefly attendants, were attacked in the hospital, most of whom had not come into contact with the patients until the latter had been subjected to the warm bath, and had changed their ordinary clothing for that of the hospital.

When a number of persons were attacked in one room there was always an interval of several days between the first and the second case, similar intervals also occurring as a rule between the succeeding attacks, so that each "tenement-epidemic" lasted several weeks.* Wherever

* In illustration of this the author gives a very striking table shewing the order in which the several members of the four families at Wilhelmsruh were attacked.

a room was inhabited by several families it was observed that the persons belonging to one family (in other words, those who wallowed on one heap of straw) were attacked sooner than those belonging to others, even though they were in the same room, so that the attacks occurred more or less in groups corresponding to families.

The author finds it impossible to regard poverty and want of food as the principal or only causes of the disease, and attributes its spread entirely to personal contagion. He states that at the commencement of the outbreak no special conditions of the kind referred to existed. The harvest had been good, and if provisions were dear, wages were relatively high. Persons were attacked under circumstances altogether removed from those of starvation, and among the very destitute it was observed that many who had previously for months spent night after night in the open air, and had remained exempt from the disease, were very soon attacked when, in consequence of the opening of the new Refuge in the town, they were brought into close relation with others.

In the distribution of the cases in those parts of the town in which they were most numerous, the only condition which seemed to have a distinct influence was overcrowding. The facts related show that during the winter in question this condition prevailed in Breslau to a frightful extent.

Observations relating to the spirilla.

Dr. Litten gives the following summary of his observations:—The spirilla of Obermeier are found only in the blood of persons affected with the disease, and present themselves invariably during the paroxysms. They are never observed during the interval of apyrexia. In more than 100 cases the spirilla were found without exception. In other patients affected with acute or other infective diseases, *e.g.*, pneumonia, erysipelas, acute rheumatism, scarlatina, measles, typhoid, variola, and particularly ague, the blood was examined with negative result. Notwithstanding, however, that the spirilla occur so constantly during the paroxysms, they cannot usually be made out immediately after the commencement of the rise of temperature. Often 24 hours and sometimes two or three days may elapse before they can be detected. Obviously the fact that in most instances they cannot be seen until some time after the first symptoms have manifested themselves, affords no proof that they did not previously exist. They invariably disappear before the critical defervescence, but not at any definite time; sometimes they may be found immediately before the temperature falls, at others they cease to be visible a day before. If, as occasionally happens, there is a transient fall of temperature in the middle of an attack (pseudo crisis), the spirilla do not disappear.

The number of spirilla found varies considerably. Whereas in some cases it is necessary to examine several preparations before a single example is met with, in others the field of the microscope swarms with them. It appears probable that the number diminishes as the paroxysm approaches its termination. The most striking fact in relation to them which presents itself to the observer is their uniformity in size and aspect. They form spiral fibrils of which the convolutions are extremely small. That this spiral appearance is not a mere result of their motion is clearly shown by the observation that after all motion has ceased it is as distinct as before. Their motions are of three kinds. In the first place, they exhibit undulations which progress along the course of each fibril, and secondly, flexions, occurring sometimes at one point sometimes at another of their axes; thirdly, they are locomotive Both the locomotive and undulatory movements are very active, and are often communicated to the corpuscular elements of the blood, so that it

sometimes happens that the presence of the bodies may be detected by the motions of the coloured disks, and particularly by a peculiar appearance of glistening undulation which courses over them, and is produced by the passage of spirilla above or below them. The other movement (of oscillation) is observed principally when the spirilla are becoming languid, as, for example, towards the end of a paroxysm. At the height of the pyrexia, on the other hand, the axis of each spiral is straighter, although the convolutions are quite as distinct. They often assume very peculiar forms, sometimes being in circles, so as to look like coloured blood disks, at others in figure of eight; at other times they attach themselves to each other in long chains which stretch over the whole field. In this last condition they do not exhibit active movement, but two spirilla so united are often seen to break away suddenly from each other, as if they were undergoing division.

It is very easy to distinguish the spirilla from the fibrils of coagulating fibrin, from which they differ both in their greater delicacy and in their mobility. Their spiral form alone is sufficient to make it easy to recognise them.

They can be best kept alive out of the body in serum. In a preparation surrounded with oil the motions continue for 24 hours. In half per cent. solution of common salt they can also be observed for several hours, but eventually their motions decline and they become altered in appearance; the wavy spiral movement continues after all locomotion has ceased. On the addition of distilled water they die instantly. Solution of caustic potash not only arrests their movements but dissolves them.

The influence of temperature on the movement was investigated with the following results:—On raising the temperature of the stage of the microscope gradually to 60° C. no effect was produced. As soon as this point was passed the movements became languid. By the time that 65° was reached they had entirely ceased. No effect was observed on cooling the preparation to the temperature of freezing. When it was kept for some time on ice the movements eventually ceased.

Observations were also made for the purpose of determining whether during the disappearance of the spirilla from the blood, *i.e.*, at the critical period of defervescence, they could be found in any other organ; the results were negative.

The presence of the large colourless transparent cells, described by Obermeier, Ponfick, and others as characteristic of relapsing fever, was observed in a good many cases. These cells are of very various form, the spheroidal being the most common. Their size varies from twice to four times, or more than four times, that of a colourless blood corpuscle. They are mostly beset with coarse strongly refracting granules. They were observed both during the paroxysms and the intermissions. In many of the cases the relative number of colourless corpuscles was obviously increased.

Addendum.

In the section of the foregoing paper relating to diphtheria, I stated that there were several pathologists who, on the ground of experiment, have come to the conclusion that the pathological process which is set up in the animal body by inoculating portions of diphtheritic concretion is not merely a mycosis, but the specific disease itself; in other words, that the distinction between diphtheria as defined nosologically and the diphtheritic mycosis is not founded on any pathological difference.

Attempts to show that in the artificial production of diphtheritic processes in animals, there is actual transmission of the disease.

Of these observers, Dr. Letzerich,* of Braunfels (Nassau), and Dr. Oertel,† of Munich, are the most important. The former is the author of several papers on diphtheria, of which I give the titles in a note. These papers contain various observations relating to diphtheria as it presents itself clinically, which serve to illustrate the intimate association of the development of microzymes in the affected parts with the morbid process; the author also records numerous experiments showing that when the disease is communicated by inoculation, its characteristics reappear in the infected animal, even those which belong to its more remote complications. Dr. Letzerich's facts lose much of their value from their not being set down with that simplicity which ought to characterize all scientific writings. His papers, moreover, contain a great deal of questionable mycology, in which the patient reader is apt to lose himself in his search after objective facts.

The mémoire of Dr. Oertel, published three years ago, also embodies anatomical and experimental investigations relating to the effect of inoculating animals with material derived from the larynx in cases of diphtheritic laryngitis in children. Like Letzerich, the author found that a disease having well-defined pathological characteristics, and in particular associated with nephritis, could be produced by such inoculation; and further, that it could be communicated from one animal to another without losing any of its distinctive features. He further showed that the disease in question, whatever were the local peculiarities given to it by the tissue in which it was ingrafted, was always a mycosis; in other words, that all the "localizations" of the disease were associated with the presence in the affected part of innumerable microzymes. As regards the agents of infection, he concluded that their presence was the only constant characteristic of the contagion, for he found that the disease could be produced by the transference to the tissues of a healthy animal of even the smallest fragment of any diseased tissue, and that all diseased tissues contained microzymes in greater or less numbers. The following short account of a series of experiments in which the disease was transmitted through five successions of animals, will enable the reader to form an estimate of the bearing and significance of the research.

The material for the first inoculation was removed about 12 hours after death from the trachea of a child, aged five, affected with laryngeal diphtheria. It consisted of the usual cellular elements embedded in a fibrinous stratum, which was infiltrated with innumerable micrococci. Of this, three fragments as large as pinheads were introduced into the

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* Letzerich, Beiträge zur Kenntniss der Diphtheritis. Virchow's Archiv, Vols. XLV., p. 327; XLVI., p. 229; XLVII., p. 516. Monographie der Diphtherie, Berlin, 1872. Die Entwickelung des Diphtheriepilzes, Virchow's Archiv, Vol. LVIII., p. 303 (1873).

† Oertel, Experimentelle Untersuchungen über Diphtherie; Deutsches Archiv für Klin. Med., Vol. VIII., pp. 242–354.

trachea of a rabbit, which died about 36 hours afterwards with the characteristic signs of croup, and exhibited in the air passages and in other organs the pathological appearances to be immediately described. With the tracheal concretions of this animal a second rabbit was inoculated, and from that a third. The latter in its turn afforded material for the inoculation of two pigeons, of which each served as a source of contagion for two rabbits. Of these two pairs of rabbits, one pair were inoculated subcutaneously, the other two being infected by the injection of the virulent material in minute quantity into the larynx. In all, the results were similar, the pathological changes and symptoms being quite as characteristic in the last of the series as in the first. The following description is that of a rabbit which received the disease in the air passages after four previous transmissions, the immediate source of infection being a pigeon.

The animal had died 96 hours after inoculation, the symptoms of laryngeal affection having lasted longer than usual. The tracheotomy wound had completely united, but underneath it there was a cavity lined with greyish material of pulpy consistence; the subcutaneous tissue of the whole body, excepting that of the back and neck, was infiltrated with sero-sanguinolent serum; and the subcutaneous lymphatic glands were enlarged and hyperæmic. From the vocal cords downwards to the middle of the trachea, a tubular, whitish, tolerably adherent membrane, of about $\frac{3}{5}''$ in length and $\frac{1}{12}''$ in thickness, covered the mucous membrane. This membrane was continuous by a narrow neck with a second membranous concretion, which extended over a great part of the larynx and the lower half of the epiglottis. In the kidneys, the epithelium of the convoluted tubes was swollen and granular, and in many instances detached. Masses of micrococci occupied the spaces between the glomeruli and their capsules, and were also distinguishable in the tubes. Capillary hæmorrhages presented themselves in great numbers in the intertubular tissue, and many of the tubes were choked with blood corpuscles. The muscular tissue—especially in the neighbourhood of the trachea—exhibited, in addition to germination of the interfascicular tissue, peculiar changes, which the author has examined into with great care. He finds that in all infected animals the contents of the muscle tubes look as if they had shrunk from the sarcolemma, and in doing so had split transversely into cylindrical fragments, between which the empty tubular sheath can often be distinguished. In the blood, micrococci and bacteria existed in enormous numbers. They were also present in the enlarged lymphatic glands, and indeed in all the affected tissues.

The reader will observe that in the series of inoculations of which a summary has been given, several links in the chain of transmissions of the diphtheritic affection from animal to animal consist of subcutaneous inoculations. In the animals so infected, the changes in the blood and tissues which may be regarded as the signs of infection, were not distinguishable from those observed when diphtheritic material was injected into the air passages, the only difference being that there was no laryngitis. In commenting on these experiments the author says:—"The "pathological process which results from the introduction of diphtheritic "material under the skin or into the tissue must be regarded as a truly "specific one, excited by a contagium identical with that which is "present in the concretions on the surface of mucous membranes. *No "such process can be induced in the animal organism by any putrescent "or decomposing material.* The general infection produced in the "animal body by diphtheritic inoculation extends centrifugally from the "seat of inoculation through the tissues, and shows no preference for

" any one organ in particular ; the participation of the kidneys, for " example, being merely a result of the existence of the infecting " material in the circulating blood."

Here all turns on the statement which I have printed in italics. To prove this, Oertel's experiments are insufficient. For the present the question whether or not the induced diphtheritic affection of rabbits is excited by a contagium identical with that of "Diphtheria," must remain open.

No. 2.

Report on the Pathology of Sheep-pox, by Dr. Klein.

Variola ovina, or sheep-pox, is a disease which, although it is not communicable to man, and possesses a specific contagium of its own, very closely resembles human smallpox, both as regards the development of the morbid process and the anatomical lesions which accompany it. This correspondence is so complete, that it cannot be doubted that the pathogeny of the two diseases is the same. That is to say, that whatever explanation can be given of the way in which the material cause or contagium produces its effects in the one case will also serve as a key to the understanding of the other. The present investigation was therefore undertaken in the confidence that the application of the experimental method to the investigation of the ovine disease, would not only yield results of value, as contributory to our knowledge of the infective process in general, but would throw special light on the pathology of smallpox.

In the following pages I propose, after giving an account of the method of investigation employed, to describe, first, the microscopical characters of the sheep-pox virus, and to compare them with those of vaccine lymph. I shall then proceed to detail the results of my investigation of the pathological changes which occur in the affected parts of the skin.

Section I.—*Method of Investigation.*

The purpose of the inquiry being to determine the nature of the pathological process, of which the cutaneous eruption is the most prominent, and probably the most important manifestation, it was necessary to examine the affected skin anatomically in all stages of the development of the disease. In order to obtain the material for this investigation, I communicated the disease by inoculation to a sufficient number of sheep. In this way I obtained specimens of skin, corresponding to each successive period in the development of the pustule, which had been removed from the animal in a perfectly fresh state. With the aid of the complete and continuous series of preparations of which I thus became possessed, I was enabled to study the process in a most satisfactory manner.

The lymph used for inoculation was obtained from two sources, one quantity having been sent to me by Professor Chauveau, of Lyons, the second by Professor Cohn, of Breslau. In some of the inoculations the lymph was first diluted with from fifteen to twenty times its volume of half per cent. solution of common salt; in others it was used undiluted. No difference was observed as regards the results between the diluted and undiluted liquids; in all cases the instrument employed for insertion was a Pravaz syringe.

Mode of inoculation.

A drop having been drawn into the steel canula of the instrument (the width of which does not exceed a fortieth of an inch), it was passed under the surface of the skin, as in subcutaneous injection. In order to avoid bleeding, great care was taken to direct the needle in such a way that it penetrated the true skin as little as possible. Before discharging it, the canula was always slightly drawn back, so as to leave the channel previously made by its point free for the reception of the expelled liquid. It will be understood that in using a subcutaneous syringe for inoculation in the manner above described, the only part of the instrument which is occupied by the liquid to be inserted is the

extremity of the steel tube; the extremely small quantity of liquid which is thus employed is ejected at the desired moment by the compression of the air behind it.

Although there can be no doubt that the nature of the pathological process in the skin is the same whether it is determined by the direct insertion of the virus into the affected part, or is conveyed to it by the circulation, yet it appeared clear that the value of the results would be impaired if the investigation extended only to the local effects produced by inoculation. I therefore felt it to be very desirable (considering it was out of the question to obtain opportunities of studying the disease as it occurs naturally) to induce it in a form resembling the natural one as closely as possible.

Natural sheep-pox, as acquired by the ordinary modes of infection, is characterised by a general eruption, the first appearance of which is accompanied by an accession of fever.

I had not only learned from my own observation that no general eruption could be induced by inoculation, but found on referring to authorities, that in those countries in which inoculation is carried on on a very large scale with a view to the protection of animals from attacks of the disease, it is extremely rare to meet with instances in which secondary pustules present themselves in addition to the primary one at the seat of insertion; it was therefore necessary to employ some other method.

I fortunately succeeded completely in producing a general eruption by introducing the sheep-pox virus into the circulating blood. Having diluted the liquid with from fifteen to twenty times its bulk of the solution of chloride of sodium already referred to, I injected it directly into the external mammary vein of a healthy sheep. At the same time that this was done I inoculated the animal in the usual way, making in all ten insertions. My object in employing both methods simultaneously was, that I might utilise the animal to the utmost. I was desirous on the one hand to induce a general infection, and thereby a general eruption; and on the other to obtain an additional supply of primary pustules for the completion of the investigations I had already made. I also thought it of great importance to compare the anatomical characters of the primary and secondary eruption in the same animal.

The injection and inoculation were performed on the 1st of April; the primary papules made their first appearance on the 4th. On the 7th several small secondary papules appeared on the lips and around the mouth, and increased rapidly in number during the succeeding three days. At the same time the eruption extended to other parts of the body, but the papules were most numerous in the axilla and on the belly. It is to be noted that the interval of time between the appearance of the first and last papules of the secondary eruption was considerable, so much so that the pustules on the lips had already formed crusts at the time that the eruption was first visible in other parts. In this case, as in the others, the primary pustules were from half an inch to an inch and a half in diameter; most of the secondary ones did not exceed a quarter of an inch, but there were a few of those which appeared latest on the chest and belly which measured half an inch or more.

Section II.—*Microscopical characters of Sheep-pox Virus.**

Clear lymph, which had been kept for several days in a sealed capiliary tube, was diluted with thoroughly boiled half per cent. saline solution.

* The description relates to a specimen of lymph sent by Prof. Cohn.

One portion of the diluted liquid having been reserved for further experimental inoculations, the remainder, which was intended for microscopical examination, was found to contain the structures represented in Fig. I. *

Fig. I.

1. Transparent masses of various sizes, containing granules, some of which are small, pale, and indistinct, others large and shining.
2. Transparent spheroid bodies arranged in small groups composed of couples or necklace-like chains; each is bordered by a sharp line as if consisting of transparent contents enclosed by a membrane.
3. Highly refractive micrococci grouped so as to form dumb-bells or sarcina or small colonies.
4. Groups of decolorised blood-corpuscles, with micrococci occupying the interstices between them.
5. Bacteria, of which the smaller ones correspond to *Bact. Termo* of Cohn.
6. Colonies of *Bacterium Termo.*
7. Micrococci mostly in couples or in small groups. Each spheroid is surrounded by a greenish envelope, or in some cases by what appears to be a thin membrane; the couples, in consequence of their being united by a transparent connecting substance, resemble rods with terminal swellings; these bodies are about twice the size of the micrococci referred to above.
8. Groups consisting of spheroids of two kinds, the larger corresponding with (2), the smaller with (3); on careful examination it is found that between the two kinds of bodies there are transitional forms, of which the characters are intermediate both as regards size and appearance. These groups appear to correspond to those figured by Cohn as microsphæra vaccinæ.

In the same preparation after it had been kept at the temperature of incubation for 24 hours, the transparent masses referred to above (1), were found to have undergone the remarkable changes shown in Fig. II.

Fig. II.

* The reference numbers on the figure correspond to those of the paragraphs.

Under a high power it is seen that they consist of spherical granules of different sizes arranged in rows, each granule being united to its successor by pale transparent substance, so that the whole appears to be made up of a feltwork of delicate granular filaments, among which separate granules are seen here and there. In preparations kept for 48 hours the larger masses are seen to have broken up into smaller ones, in which the individual fibrils can be more easily traced, while the granules themselves have assumed characters which correspond to those of the micrococci (3), and pale spheroids (2) seen in the fresh lymph. This conversion of the granules contained in the masses into spheroids and micrococci, goes on for some days, so that these bodies multiply very considerably in the liquid.

Examination of perfectly recent Lymph.

A drop of lymph obtained on March 24th from a pustule of an animal that had been inoculated March 10th, was examined microscopically without dilution, and was found to contain, in addition to granular pus corpuscles and coloured blood corpuscles, numerous small highly refractive granules, either isolated or in couples, which exhibited molecular movement. After having been kept at 32° C. for 27 hours, it exhibited the following structures, (Fig. III.) in addition to those already mentioned :—

Fig. III.

* 1. Pus corpuscles, of which the substance has become swollen and transparent, each containing from two to six homogeneous slightly refractive spheroids. These bodies, of which the diameter is less than half that of a coloured blood corpuscle, are also seen in considerable numbers in the free state ; they possess a slightly greenish colour, and are perfectly homogeneous, differing in both of these respects from nuclei of pus corpuscles. When not enclosed in pus corpuscles they occur either isolated or in couples ; in form they are usually spheroidal, but are occasionally oblong, showing a more or less marked constriction in the middle. Along with these, other forms exist, in which it can be made out that the body, if a spheroid, consists of a highly refractive smaller

The numbering of the paragraphs corresponds as before, to that of the Fig. (III.)

granule (micrococcus) contained in a transparent envelope ; or if oval or rod-like, of two such granules held together in a similar manner. Between these and the free micrococci to be immediately described, it is easy to observe the transition.

2. By the repeated division of the spheroids above described, the dumb, bells and necklaces shown in the figure * are formed ; the constituent micrococci of the necklaces are held together by a connecting transparent substance. Sometimes they are all of the same size, but more frequently one is larger than the rest, and presents the appearance of a greenish transparent pear-shaped body, which may or may not contain a highly refractive granule. The necklaces, which have grown to a great length and have become much convoluted, are apt to break up into shorter chains, forming
3. Groups from which the free ends of the filaments project. By the coalescence of these groups of convoluted chains, colonies, consisting of micrococci closely packed together, are produced, which correspond in appearance to the Zooglæa of Cohn ; these colonies are often connected together by
4. Long filaments, some of which still show a necklace-like structure, while others are apparently smooth and homogeneous. Twenty-four hours later the colonies were found to have increased in number and size; some of the individual micrococci had also undergone changes, having not only acquired larger dimensions, but having assumed the appearance represented in Fig. I. (7.)

From the appearance above described I conclude that the highly refractive spheroid is the only form that can be regarded as characteristic of the lymph of sheep-pox in its active condition.

Section III.—*Anatomical Investigation of the Changes which occur at the Seat of Inoculation.*

1. The development of the primary pock may be divided into three stages, of which the first is characterised by progressive thickening of the integument over a rapidly increasing but well-defined area; the second, by the formation in the rete Malpighii of vesicular cavities containing clear liquid (the "cells" of older authors), in which sooner or later organic vegetable forms are developed ; the third, by the impletion of these cavities with pus-corpuscles. It is to be noted that the division into stages is less marked than in human small-pox.

2. The process commences in the rete Malpighii and in the subjacent papillary layer of the corium—in the former, by the enlargement and increased distinctness of outline of the cells, and by corresponding germinative changes in their nuclei; in the latter, by the increase of size of the papillæ, and by germination of the endothelial elements of the capillary blood-vessels.

3. It is next seen that the interfascicular channels (lymphatic canaliculi) of the corium are dilated and more distinct; that the lining cells of these channels are enlarged and more easily recognised than in the natural state; and that in the more vascular parts of the corium, the channels are more or less filled with migratory or lymph corpuscles. At the same time, the lymphatic vessels, of which the canaliculi are tributaries, can be readily traced, in consequence of their being distended with a material which resembles coagulated plasma.

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* The particles forming the necklaces present the appearance of solid bodies ; this is not well shown in the engraving.

4. About the third day after the appearance of the pock, the contents of the dilated lymphatics begin to exhibit characters which are not met with in ordinary exudative processes. These consist in the appearance in the granular material already mentioned, of organised bodies, which neither belong to the tissue nor are referable to any anatomical type—viz. of spheroidal, or ovoid bodies having the characters of micrococci and of branched filaments. In Fig. IV. a lymphatic vessel of the corium

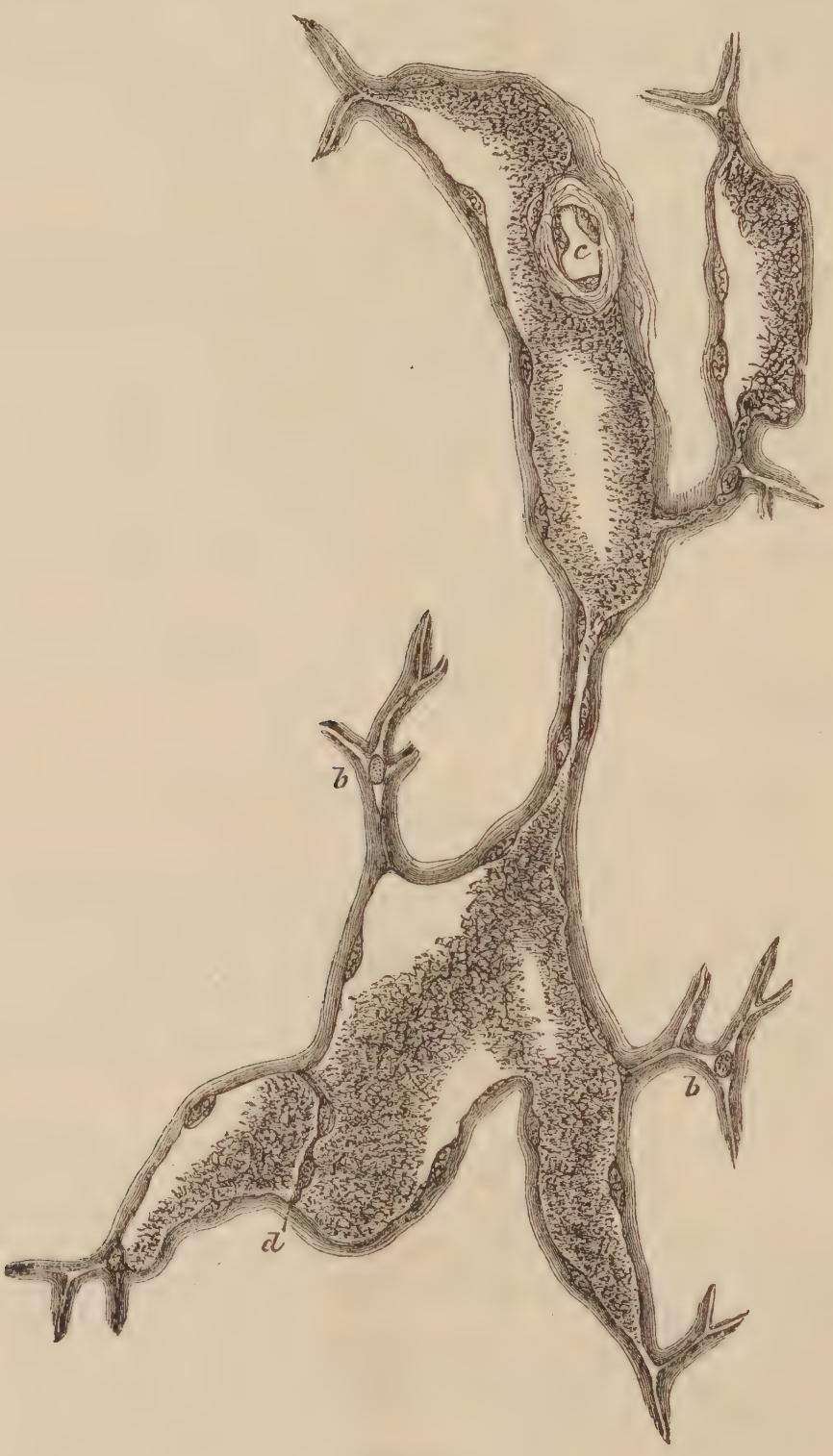


Fig. IV.

is seen in section filled more or less completely with micrococci. In some parts the spheroids are aggregated in the zooglæa form, so that the mass appears granular, in others it presents a filamentous aspect, the spheroids being in necklaces or chaplets. In the upper part of the figure a blood-vessel (*c*) is shown in section which is sheathed in the lymphatic channel, at (*d*) a valve is shown with masses of micrococci on

either side of it; (*bb*) indicate interfascicular channels containing connective-tissue corpuscles. In Fig. V. a similar lymphatic vessel of the

Fig. V.

corium is represented, of which the contents have the characters of a mycelium, and consist of branched filaments. In Fig. VI. the same

Fig. VI.

structure is seen, but the interlacing of the filaments is so dense that the whole presents the aspect of a felt-like mass.

5. The process thus commenced makes rapid progress. After one or two days the greater number of the lymphatics of the affected part of the corium become filled with the vegetation above described; and on careful examination of the masses, it is seen that they present the characters of a mycelium, from which necklace-like terminal filaments spring, each of which breaks off at its free end, into conidia. In most of the filaments, a jointed structure can be made out, and in the larger ones, the contents can be distinguished from the enclosing membrane by their yellowish-green colour.

The necklace-like filaments are well seen in Fig. VII. They are

Fig. VII.

contained in a lymphatic vessel of the corium similar to those represented in Figs. IV., V., and VI.

Separated conidia, in a state of germination, are shown in Fig. VIII., as seen under a much higher power.

Fig. VIII.

6. At the same time that these appearances present themselves in the corium, those changes are beginning in the now much thickened rete Malpighii, which are preparatory to the formation of the vesicular cavities already mentioned. By a process which I propose to designate horny transformation, having its seat in the epithelial cells of the middle layer of the rete Malpighii, a horny expansion or stratum appears, lying in a plane parallel to the surface, by which the rete Malpighii is divided into two parts, of which one is more superficial, the other deeper than the horny layer. Simultaneously with the formation of the horny layer the cells of the rete nearest the surface of

the corium undergo very active germination, in consequence of which the interpapillary processes not only enlarge, but intrude in an irregular manner into the subjacent corium. At the same time, the cells immediately below the horny stratum begin to take part in the formation of the vesicular cavities, some of them enlarging into vesicles, while others become flattened and scaly, so as to form the septa by which the vesicular cavities are separated from each other.

The whole of this process is well shown in Fig. IX.,* which repre-

Fig. IX.

sents a vertical section through the central portion of a primary pock on the eighth day.

The stratum corneum is unaltered. The newly-formed horny layer by which the rete Malpighii is abnormally split into a superficial and a deep layer, is seen to be formed by the transformation of the cells which lie next to it on its superficial aspect. On its deep surface are cells

* (*a*) Stratum corneum, (*b*) superficial layer of *rete Malpighii*, (*c*) deep layer of do., (*d*) newly-formed horny layer, (*e*) superficial or papillary layer of corium.

which are much enlarged and contain vacuoles; they present the appearance to which pathological histologists frequently apply the term dropsical. It is by the subsequent dilatation of these enlarged cells that the vesicular cavities ("cell" of the older authors) are formed; between them the septa are seen composed of flattened and scaly cells which, as above described, extend from the horny layer towards the interpapillary processes. The drawing further shows the remarkable changes which take place in the papillary processes themselves simultaneously with the formation of the horny layer. In consequence of the very active germination of the cells of which these processes consist, they send down conical or cylindrical sprouts into the corium; it sometimes happens that the end of one of these sprouts is cut off, so that it presents itself in the section as an island of cells (*h*) surrounded by the tissue of the corium.

7. The vesicles, once formed, increase in size and number. Originally separate, and containing only clear liquid, they coalesce, as they get larger, into irregular sinuses, and are then seen to contain masses of vegetation similar to those which have been already described in the lymphatic system of the corium—with this difference, that the filaments of which the masses are composed are of such extreme tenuity, and the conidia are so small and numerous, that the whole possesses the characters of zooglæa rather than of mycelium. There appears to me to be little doubt that these aggregations are produced in the same way as the others, viz., by the detachment of conidia from the ends of filaments. The characters of the vegetation contained in the vesicular cavities are well shown in Fig. X. The contents of a single vesicle, as

Fig. X.

seen under a much higher power, are represented in Fig. XI. In the earlier stages of the process the cavities contain scarcely any young cells. Sooner or later, however, so much of the rete Malpighii as lies between the horny stratum and the papillæ becomes infiltrated with migratory lymph-corpuscles. The process can be plainly traced

in the sections. At the period of vesiculation, *i.e.*, at a time corresponding to the commencement of the development of the vesicles in the

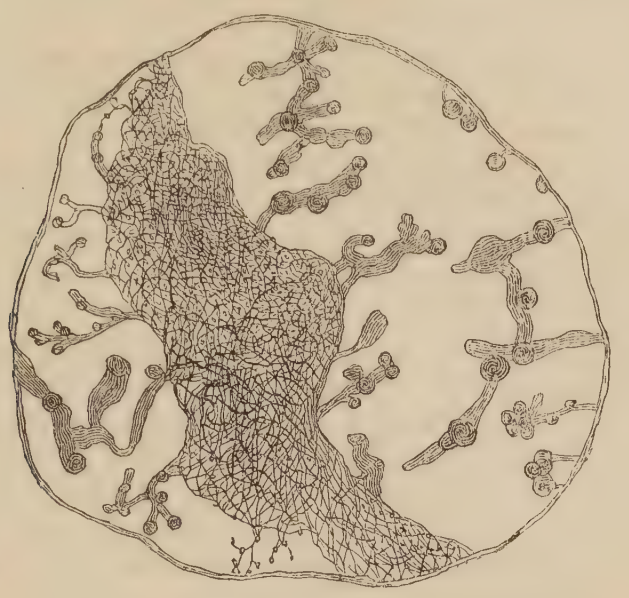


Fig. XI.

rete Malpighii, the cutis (particularly towards the periphery of the pock) is infiltrated with these bodies. No sooner has the coalescence of the vesicles made such progress as to give rise to the formation of a system of intercommunicating sinuses, than it is seen that the whole of the deep layers of the rete Malpighii become inundated (so to speak) with migratory cells, which soon find their way towards the cavities, and convert them into microscopical collections of pus-corpuscles, the formation of which is proved to be due to migration from the corium, not only by the actual observation of numerous amœboid cells *in transitu*, but by the fact that the corium itself, before so crowded with these bodies, becomes, as the pustulation advances, entirely free from them.

Section IV.—*Anatomical Investigation of the General Eruption.*

The anatomical characters of the secondary pocks are substantially the same as those of the primary, the most prominent features being thickening of the rete Malpighii and œdema of the corium, combined with the presence of lymph corpuscles around the blood-vessels, with similar corpuscular infiltration of the lymphatic canalicular system in the neighbourhood.

In general the stage of pustulation is reached more rapidly in the secondary pustules than in those which are the direct result of inoculation. Thus, in the eruption on the lips the contents of the vesicular cavities became purulent not later than the third or fourth day; after the appearance of papules on the chest the development was more tardy. The infiltration of the cutis and papillary tissue was greater towards the periphery than towards the centre, especially in those pustules that had lasted longest and exhibited most distinctly a central depression. There were also considerable differences as regards the changes which, in the primary pustule, result in the splitting of the rete Malpighii into two layers. The peculiar transformation which in the primary pustule goes on to such an extent as to result in the formation of a horny layer, which is of such thickness and so well marked that it can be distinguished in the section by the naked eye, is represented

in the secondary pustules by a change of the same kind, which, however, is very partial in its extent and distribution, and affects only a few cells of the middle layer of the rete. Connected with this there is a considerable difference in the mode of the formation and the arrangement of the vesicular cavities. They appear in great number simultaneously in the middle layers of the rete Malpighii, and are generally found much nearer the corium than in the primary pocks. It is further to be noticed as a collateral fact that in consequence of the number and progressive dilatation of the vesicles at the expense of the deepest layers of the rete, the inter-papillary processes become obliterated, so that the corium is marked off from the rete by a line which is almost as even as it is in sections of the natural skin. As a further result of the distinction of the vesicles, it is sometimes seen that the deepest cells of the rete are altered in form by compression. As regards the distribution of the vesicles in the secondary pustule, it is to be noticed that even in those pocks that exhibited a marked central depression, the most numerous and well-developed vesicles were found towards the centre; this is clearly inconsistent with the supposition that the depression is caused by the disappearance of previously existing vesicles.

As has been already stated, the vegetations contained in the lymphatics of the corium, as well as those occupying the vesicular cavities, presented the same characters as those which have been already described in the previous section. In some vesicles the mycelium is embedded in a finely granular matrix which is to be regarded as coagulated plasma; in others the matrix is almost homogeneous, and is stained slightly by carmine and hæmatoxyline. The mycelium itself, as well as the spores which devolve from it, exhibit a bright and shining appearance. Eventually the mycelium is transformed by a process of rapid fructification into a mass of micrococcus resembling zooglæa, the characters of which are not to be distinguished from those of the similar masses which are met with in the primary pustules when examined at a later stage of their development.

No. 3.

Report of Anatomical Researches into the Lymphatic System and its relation to Tubercle, by Dr. Klein.

Part I.—Lymphatic System of the Serous Membranes.

A. *Normal Anatomy.*

1. *The Endothelium of the free surface of the Serous Membranes* in the normal condition is composed not only of a layer of flattened cell-plates, but in some places, hereafter to be described, of cells, which appear polyhedral, club-shaped, or like short columns ; their substance is a distinctly granular protoplasm, even in the fresh condition, and their nucleus is either marked by a constriction, or completely divided into two. Endothelial cells of this kind may be called *germinating endothelium*, for they are in constant prolescence, giving origin to lymphoid cells, which gradually become detached from the free surface.

Such germinating endothelial cells are found in smaller or larger groups on the fenestrated omentum, on the large and small trabeculæ,

Fig. 1.

Germinating endothelium of tracts freely projecting over the surface of the pleura mediastini of a healthy cat. Preparation stained with nitrate of silver. Magnifying power of about 300.

constituting that membrane ; they contain large blood-vessels and fat tissue. On these large trabeculæ nodules and cord-like structures are found more or less projecting over the free surface, which are covered

generally on one side only with germinating endothelium. This kind of endothelium is seen not only in the fenestrated omentum of the dog, cat, guineapig, rat, and monkey, but also on the omentum of the rabbit, which is very little, or not at all, fenestrated; here the germinating endothelium is also found on the surface of certain patches of structures of different sizes, which are isolated or in groups, or form a continuous cord. The pleura mediastini of dog and cat are especially rich in nodules and cords, covered with germinating endothelium.

The mesogastrium of frogs (especially of female frogs in the winter months) exhibits also groups of germinating endothelium, the cells of which are often provided with delicate cilia on the free surface.

So also the mesentery and the septum cisternæ lymphaticæ magnæ are in some places covered with small groups of germinating endothelium; the germinating cells in the latter membrane being also in female frogs provided with cilia, especially during the spawning season.

On the peritoneal surface of the diaphragm there are stripes of small germinating endothelial cells covering certain lymphatic vessels, which will be afterwards described as radiating straight lymphatic capillaries.

2. *The Cellular Elements of the Matrix of the Serous Membranes* generally consist of (*a*) flattened, more or less branched, isolated cells; and (*b*) migratory cells. In some places, however, the flattened cells

Fig. 2.

Silver preparation of the pencilled pleural surface of centrum tendineum of a guineapig suffering from slight chronic inflammation.

a, Lymphatic capillaries. *b*, Lymph-canalicular system lined by connective-tissue corpuscles.—Magn. × 300.

are so close to one another, and so little branched, that they resemble a group of endothelial cells. The first lie in a preformed system of spaces (the lymph-canalicular system of Recklinghausen), in such a manner that the nucleated part of the flattened cell occupies a lacuna, while their branches lie in a corresponding system of canals,—the anastomosing canals

between neighbouring lacunæ. Through this lymph-canalicular system the amœboid cells pass, *i.e.* migrate. The first kind of connective-tissue corpuscles are to be regarded as an imperfect, *i.e.* disconnected endothelial lining of the lymph-canalicular system. This fact is brought out in a striking manner when the lymph-canalicular system happens to be dilated either naturally or after injection.

In certain parts of the serous membrane, especially in the vicinity of the larger blood-vessels, the connective-tissue corpuscles undergo remarkable changes, being converted into true lymphatic glandular structures. These structures are generally at first small, and not much elevated above the surface of the membrane; but as they grow they become more elevated, presenting the appearance of larger or smaller nodules or cords. They are very numerous in the omentum of the rabbit and of other mammalian animals (cat, dog, rat, guineapig, and monkey), but most numerous in the pleura mediastini of the cat and dog.

They may be described as follows :—

(*a*) Patches and nodules, the matrix of which consists of groups of ordinary flattened branched cells, which on the one hand multiply by division, so that the organ increases in size, and from which on the other hand lymphoid cells originate. The branched and lymphoid cells lie in the lymph-canalicular system. At an early stage of development they do not contain blood-vessels; at a later period they possess a special system of capillary blood-vessels. By growing in length they join one another, and form cords and tracts.

Fig. 3.

From a fresh oedematous omentum of a guineapig suffering from chronic peritonitis.
a, Lymphatic vessel. *c*, Its endothelial wall. *b*, Peri-lymphangeal nodule. *d*, Its capillary blood-vessels. *e*, Lymphoid cells.—Magn. × 300.

(*b*) In many of these structures the branched cells of the matrix are gradually converted into a delicate reticulum, in the meshes of which the lymphoid cells are contained; at this stage they are generally provided with blood-vessels, and entirely resemble lymph follicular structures.

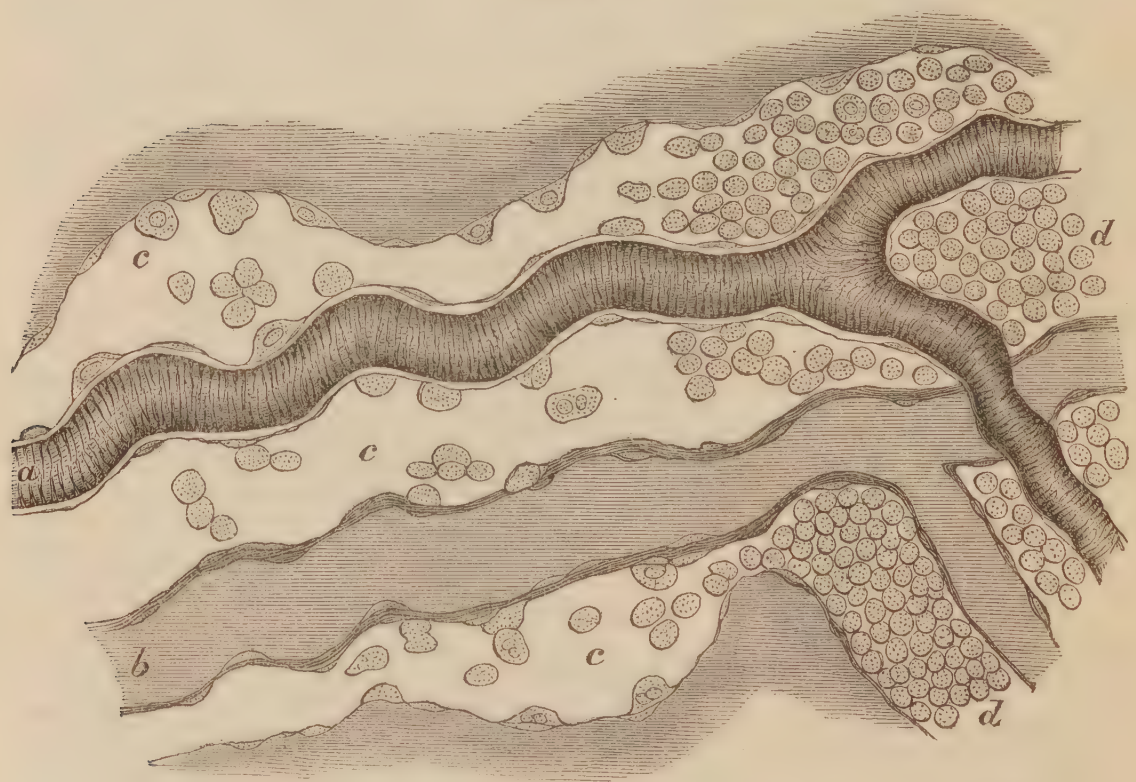
(*c*) There are, however, other nodular and cord-like structures which do not take their origin from the ordinary connective-tissue corpuscles of the matrix of the serous membrane, but which develop within lymphatic vessels in connection with their lining endothelium. These nodules and cords possess the same structure as those mentioned in (*b*); *i.e.* they resemble true lymph follicular structures.

The first two kinds of nodules and cords are to be designated as peri-, the third as nedo-lymphangeal structures; for, as will afterwards be seen, all three kinds are developed in connection with the wall of lymphatic vessels; but the two first grow from the outside, and the third from the inside.

Fat tissue stands in close relation to the perilymphangeal nodules; for fat cells are developed not only from connective-tissue corpuscles, but the fat tissue of the serous membranes develops, as a rule, from the perilymphangeal nodules, the matrix of which is converted into fat cells. It is therefore evident that in a given serous membrane the number of lymphangeal structures stands in inverse relation to that of fat tracts. The proportion in which perilymphangeal nodules are converted into fat tissue differs considerably in different serous membranes. Thus, *e.g.* this change takes place considerably in the omentum and mesentery of the cat and dog; whereas it occurs very little in the pleura mediastini of these animals. In general it may be stated that the tendency to this change is greatest in the mesentery and least in the pleura mediastini.

3. *The Lymphatic Vessels of the Omentum.*—A. In the rabbit these are differently arranged in different parts. In the neighbourhood of the great curvature of the stomach and in the portion of omentum covering its pyloric end, where there is a rich network of lymphatics, they are related to the large blood-vessels in such a way that a group of the latter has two lymphatics, one on each side, communicating with one another by transverse and oblique lateral branches. The lymphatics are also distributed independently of large blood-vessels, chiefly between and around the lymphatic patches mentioned above. Concerning their structure, there is little to add to what is already known. They are wide vessels, the walls of which consist of one layer of endothelial plates; these

Fig 4.



From an oedematous omentum of a monkey suffering from chronic peritonitis.
a, Artery. *b*, Vein.
Both these vessels invaginated in a lymphatic *c*, which contains numerous lymph-corpuscles.—Magn. × 300.

latter, in the large lymphatics which accompany the blood-vessels, being of a short, spindle-like, and somewhat sinuous outline. They possess valves and corresponding sacculated dilatations, and are to be considered as the main lymphatic trunks.

The other lymphatics, distributed in the tissue, are not different in calibre or in the form of the endothelium; but they are either without valves, or have them only here and there, while they are provided with

sinus-like dilatations springing from their sides. These are to be considered as the lymphatic capillaries. In the portion of omentum covering the anterior wall of the stomach, the conditions are somewhat different. Here the lymphatics are only seen in company with the blood-vessels, which in this part are rarely large, the vessels of common occurrence being the smaller arteries and veins, the courses of which are often isolated for considerable distances. The arrangement of the lymphatics may be summed up as follows:—A single lymphatic vessel is accompanied on one side frequently by a vein, sometimes also by an artery; the lymphatic possesses at certain points saccular dilatations, and receives occasionally a lateral branch. These vessels have mostly a sinuous endothelium. Or, secondly, a blood-vessel is accompanied on each side by a lymphatic; these are disposed exactly as in the former case, and communicate by cross branches. Finally, it occurs not unfrequently that the blood-vessel runs within a lymphatic, *i.e.* the blood-vessel is invaginated.

B. The lymphatic vessels of the omentum and pleura mediastini of other mammalian animals (as in the cat, dog, rat, guineapig, and monkey) have an arrangement similar to that in the same organs of the rabbit, *i.e.* they accompany the larger blood vessels, either singly or more frequently in pairs, and are in a few cases of an invaginating character. As regards their structure, they appear to consist simply of a wall composed of a single layer of endothelial cells.

Two points concerning the origin of lymphatic vessels from the tissue are of great importance. (*a*) The lymphatic vessels are in continuity with the connective-tissue corpuscles in such a way that on the one hand the *lumen* of the vessel is in direct communication with the lymph-canalicular system, and on the other hand the *endothelium* of the vessel is in unbroken continuity with the branched connective-tissue corpuscles occupying that system. Where the connective-tissue corpuscles form the nodules and cords above described as perilymphangeal structures, the surrounding lymphatic vessel is seen to be in such a relation to them that the lumen of the lymphatic vessel loses itself in the labyrinth of lymph spaces in which the branched cells of the matrix of the peri-lymphangeal structures are situated; and that these latter cells are in continuity with the endothelial wall of the lymphatic. So that properly speaking these peri-lymphangeal structures appear to be situated only in the wall of the lymphatic vessel. (*b*) The invaginating lymphatic vessels have an important relation to the origin of those lymph-follicular nodules and cords that we designated above endolymphangeal structures. A lymphatic vessel invaginating a vein is seen to become dilated into a wide sac where the vein receives its capillary tributaries, which are themselves invaginated in that sac; or a lymphatic vessel that accompanies a vein possesses a blind saccular dilatation, in which the system of capillary vessels belonging to that vein is contained like the glomerulus of a Malpighian corpuscle of the kidney. In either case it may happen that the lumen of the invaginating lymphatic vessel gradually loses itself in a labyrinth of spaces by the growth of a reticulum of branched cells within its cavity, and in connection with its endothelial wall; the meshes of the reticulum are occupied by lymphoid cells originating from that reticulum. In this way we are enabled to understand the formation of true lymph-follicular nodules and cords, to which from their being within a lymphatic vessel the word endolymphangeal may be applied.

4. *The Lymphatic Vessels of the Mesentery.*—The mesentery possesses two kinds of lymphatic vessels first, vessels which run from

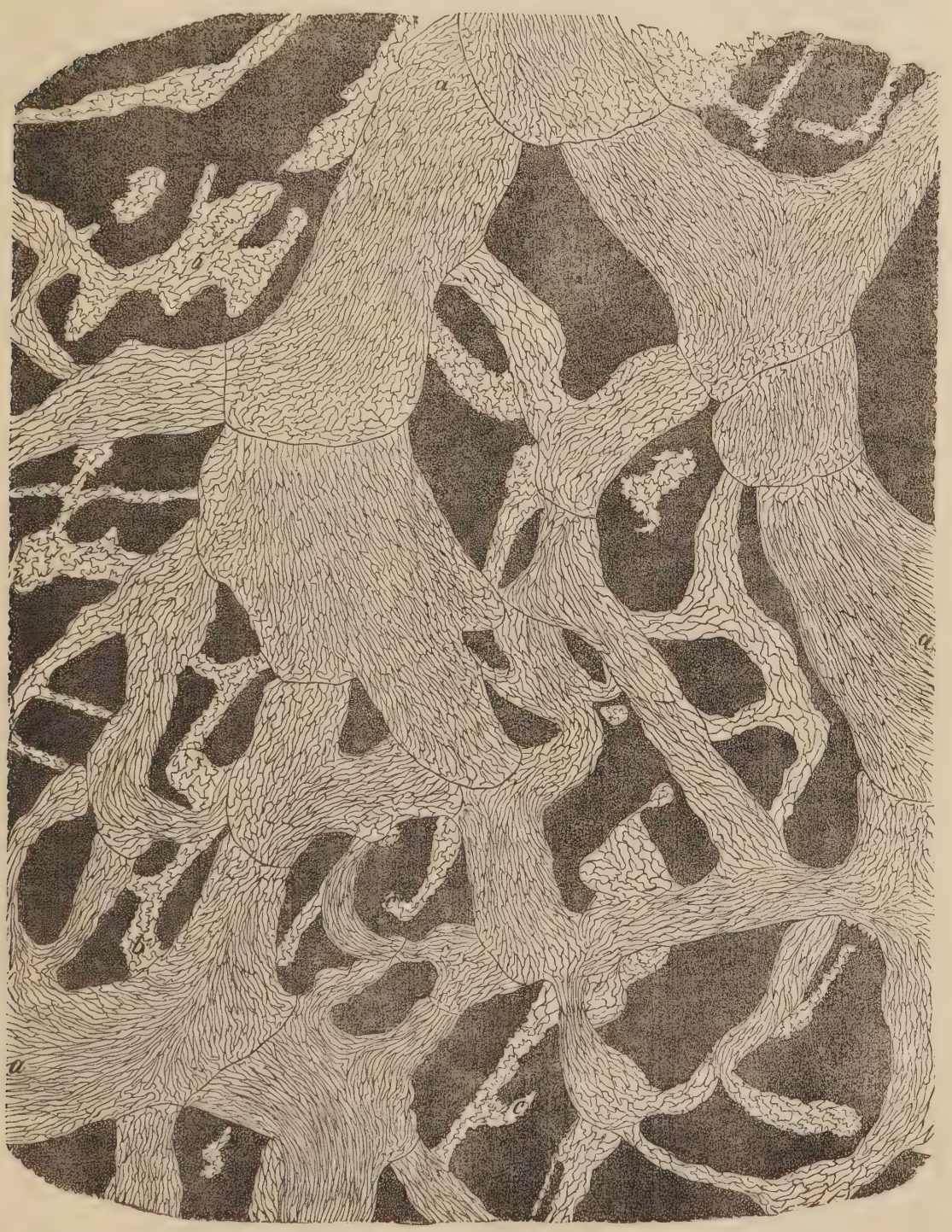
the intestine to the roots of the mesentery in order to enter the mesenteric glands ; and, secondly, vessels which belong to the mesentery itself. The vessels of the first kind are situated in the chief trabeculæ of the mesentery, along with the large blood-vessels, which run to and from the intestine. These lymphatic vessels are distinguished by the number of their valves and by their continuous well-developed circular muscular coat. Their endothelium is spindle-shaped, like that in the lymphatic trunks in general. The vessels of the second kind are : (*a*) large lymphatic vessels, which like the others accompany the larger blood-vessels. They may be single, but are more frequently in pairs ; in the former case the lymphatic is situated between the arterial and the venous trunk ; in the latter the artery and the vein lie both between the two lymphatics. They anastomose with each other by a few wide branches. Here and there they possess a valve ; they have no muscular coat, their wall consisting only of one layer of endothelium, which is more or less elongated and spindle-shaped. In many places the breadth of the endothelial cells is not much inferior to their length. Lymphatic vessels of this kind are often found to invaginate an arterial or venous trunk for a longer or shorter distance, especially with their large saccular dilatations. These dilatation-sinuses are constantly found in the lymphatics of mammals, as well as in those of frogs. Especially in rana temporaria, and in bufo, I have come across not a few mesenteries in which, instead of lymphatic vessels accompanying blood-vessels, there was a system of lymph-sinuses, situated behind each other and communicating with each other by small openings. This is determined principally by the arrangement of the ground-substance, for in the cases just referred to there were large trabeculæ in which the blood-vessels and nerve-trunks were imbedded, and from which small branches sprang at regular intervals, anastomosing with each other so as to form a mesh work. Seen from the surface it looked exactly like a fenestrated membrane. The meshes were occupied by the lymph-sinuses above mentioned. In frogs not only a single blood-vessel or nerve-trunk, but sometimes the whole trabeculæ containing the blood-vessels and the nerve-trunk may be invaginated in a lymphatic vessel.

(*b*) Besides those lymphatics which are found in and close around the chief trabeculæ of the mesentery, there are others which belong to that portion of the membrane which is intermediate to these trabeculæ. These are more or less wide vessels, having no valves ; their wall is a single layer of rhombic endothelium with sinuous outlines. They correspond to the structures generally called lymphatic capillaries, and generally accompany the smaller veins. In the pencilled silver-stained mesentery of the dog, cat, monkey, and frog, they are very easy to demonstrate. They anastomose with each other, forming a wide net-work, and like the vessels previously mentioned, are provided with dilatation-sinuses. In those mesenteries where there is little fat tissue, and where consequently it can be easily seen that the lymphangeal *plaques* and tracts consist of branched cells, it is also evident that these patches and tracts are always accompanied by one or more lymphatic vessels.

5. *The Lymphatic System of the Centrum Tendineum of the Diaphragm.*—From Recklinghausen's experiments it is well known that the very abundant lymphatics of the centrum tendineum are distinguishable into lymphatic trunks and lymphatic capillaries, the former being provided with valves and lined by spindle-shaped endothelium, the latter having no valves, having a lumen of variable size, numerous excavations, and sinuous endothelium. Recklinghausen states that some of the lymphatic capillaries of the pleural serosa have a blind end

like a horn curved inwards, at which point they lose themselves in the lymph canalicular system. Ludwig and Schweigger-Seidel have further shown that the lymphatics of the pleural serosa of the centrum tendineum of the rabbit communicate with lymphatic vessels, which run between the tendon-bundles. As these bundles have on the abdominal surface a radiating and on the pleural a circular arrangement, the two lymphatics which run between them have respectively a radiating and a circular course, and the former are called by Ludwig and Schweigger-Seidel superficial, the latter, deep lymphatic channels. My researches

Fig. 5.



Silver-stained preparation of a pencilled normal centrum tendineum of rabbit.

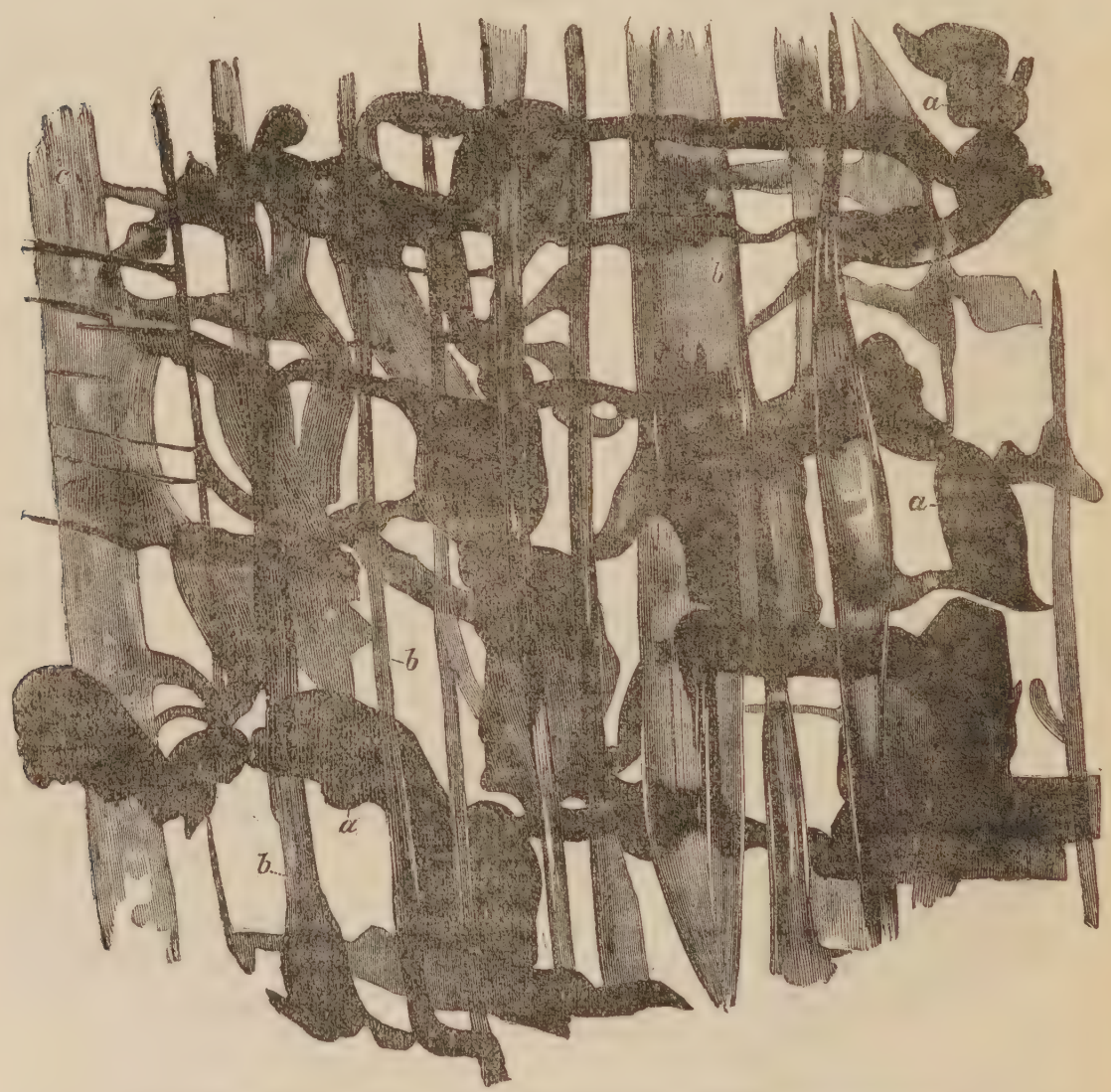
a, Large lymphatics with valves. *b*, Lymphatic capillaries. *c*, Lymphatic capillaries dipping down between the tendon-bundles.—Magn. × 50.

teach me the following facts respecting the distribution of the lymphatics in the centrum tendineum of the rabbit. They are for each half arranged in two systems, an anterior and posterior. The vessels of the anterior system are distributed upon the outer and anterior (that is

larger) portion of the anterior quadrant, and upon the outer (that is smaller) portion of the posterior quadrant. Those of the posterior system are distributed upon the inner posterior (that is smaller) portion of the anterior and upon the inner (that is larger) portion of the posterior quadrant. (The relative number of lymphatics is greater in the posterior quadrant than in the anterior). The efferent trunks of the anterior system course along the *pars costalis* of the diaphragm, and usually unite on the posterior surface of the xiphoid cartilage on each side, with one or two large vessels, which run along with the mammary vessels towards the sternal gland. On their way they join with those lymphatics that come from the intercostal spaces.

The efferent trunk of the posterior system is single on each side; it mounts obliquely towards the middle line, and opens into the thoracic duct near the point where the latter emerges from the diaphragm. Each system communicates with the corresponding one of the other side by a few large vessels. The vessels of each system have the following characters: the large vessels possess valves and a wall consisting of spindle-shaped endothelium; they are situated chiefly between the pleural serosa and the tendons, or in the depth of that serosa. The capillaries that run into these vessels have no valves, are provided with

Fig. 6.



Natural injection of the lymphatics of the centrum tendineum of rabbit.
a, Large lymphatics of the pleural side. *b*, Straight lymphatics of the abdominal side.—Magn. × 80.

irregular excavations, and their wall consists of sinuous endothelium. There are two kinds of capillaries: *a*, those that lie chiefly in the pleural serosa, and are of a variable breadth; *b*, those that lie between the

tendinous tissue. These run, consequently, in a straight course in two directions, so that we may distinguish deeper capillaries running in a circular direction and more superficial capillaries running in a radial direction. These two kinds of straight lymphatic capillaries generally communicate where they cross each other, by an extremely short branch at the points where their walls come in contact; or a superficial straight capillary may sometimes be seen to bend at a right angle, so as to continue its course as a deep straight capillary. The deep straight capillaries represent the vessels of communication between the superficial straight capillaries and the lymphatic vessels that lie in the pleural serosa. As a rule, the deep straight capillaries, before they join a lymphatic trunk, run a short distance, in a more or less wavy course, between the tendon and the pleural serosa.

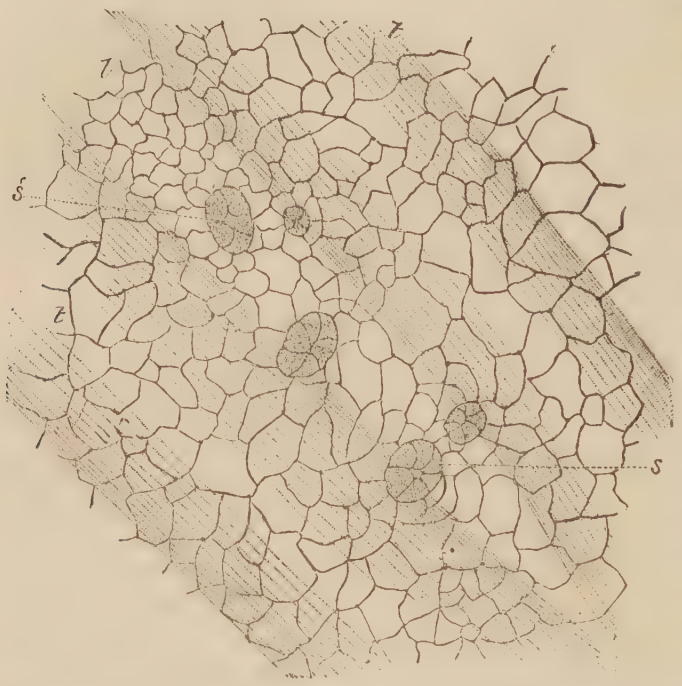
The question which now presents itself is, What is the physiological value of the straight lymphatic vessels? Ludwig and Schweigger-Seidel attribute to them an important *rôle* in absorption. According to these authors they are widely dilated when the diaphragm is in the position of inspiration and its muscles and tendons are in action, whereas they are compressed during expiration, the lymphatics of the pleural surface of the diaphragm being in the opposite condition. Consequently, the diaphragm acts like a pump on its lymphatics. (The free communication of the lymphatics with the peritoneal cavity, by means of stomata, will be discussed hereafter.) This, however, is not the only way the straight lymphatics act. We have mentioned already that the lymphatics of the diaphragm are arranged in an anterior and a posterior system, the former discharging itself by trunks that run towards glands behind the sternum, the latter by a wide short trunk that runs directly into the thoracic duct. Now the straight lymphatic capillaries are the vessels by means of which these two systems communicate, and this is their chief function. As the deep straight lymphatic capillaries and the superficial ones represent, as we have seen, only one category of vessels, we are justified in saying that this category of lymphatic capillaries discharges itself in two directions: one freely into the thoracic duct, and a second less freely towards the sternal gland. We shall see afterwards that the straight lymphatic capillaries, both deep and superficial, are in free communication with the peritoneal cavity, by means of vertical lymphatic canals (stomata of authors); consequently we have to correct the diagram of Ludwig and Schweigger-Seidel representing the lymphatics as a single pump, to a diagram of a pump with two cylinders, one corresponding to the pleural vessels of the anterior system, the other to those of the posterior system, while the pipe connecting the two cylinders corresponds to the straight capillaries, and the piston tube to the vertical lymphatic canals. It must be borne in mind, however, that the two cylinders act simultaneously.

The superficial straight lymphatic capillaries are not the only capillaries that are to be met with on the abdominal surface of the centrum tendineum of the diaphragm of rabbits and guineapigs. Near the median line of the anterior quadrants, and near the large blood-vessels that pass through the diaphragm, there exist lymphatic capillaries of a winding course, provided with numerous dilatation-sinuses. They are in communication with the straight lymphatic capillaries. In some places two neighbouring superficial straight capillaries anastomose with each other by means of a transverse branch provided with sinuses, which belong to the peritoneal serosa.

6. *Relation of the Lymphatic Vessels to the surface of the Serous Membranes.*—The lymphatic system of the serous membranes communicates with the free surface, *i.e.* with the serous cavity, in two ways, (*a*) by

means of true stomata, *i.e.*, vertical lymphatic channels, lined by a special layer of endothelium, and leading into the lumen of a superficial lymphatic vessel, or (*b*) by means of small holes,—discontinuities between the surface-endothelium of the serous membrane, leading into a superficial simple lymphatic lacuna. By this term is understood a cavity lined only on one side by endothelium. Such a cavity may be supposed to be formed by the confluence of a number of adjoining lacunæ. Both kinds of stomata are bordered on their free surface by germinating endothelial cells. The first kind is met with in great numbers upon the straight lymphatic capillaries on the peritoneal surface of the central tendon; on the above-mentioned excavations and sinuses of the lymphatic capillaries of the peritoneal serosa of the central tendon; and on the lymphatic capillaries and lymph sinuses in the omentum and pleura mediastini of

Fig. 7.



Silver preparation of the peritoneal surface of the centrum tendineum of the diaphragm of rabbit.

l, Endothelium covering the serosa over the tendon-bundles. *s*, Stomata over the straight lymphatics, *l*. —Magn. × 160.

Fig. 8.

Silver stained preparation of the septum cisternæ of frog; showing stomata.

A, Viewed from the peritoneal surface. B, Viewed from the cisterna.—Magn. × about 160.

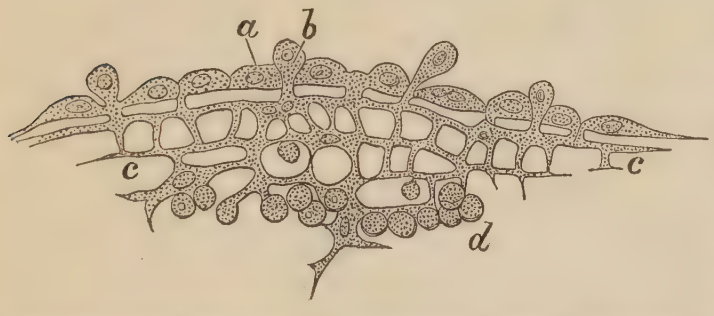
all animals. These stomata are less numerous on the lymphatic capillaries of the mesentery. The well-known stomata on the septum cisternæ lymphaticæ magnæ of frogs first described by Dgoiel and

Schweigger-Seidel, belong to this category. In contradiction to the assertion of these authors it must be remarked that the stomata in question represent vertical lymphatic canals, lined by a special layer of more or less polyhedral endothelial cells of a germinating character, and that they are not merely discontinuities between the endothelial cells having nuclei directed towards the aperture.

But there is also another kind of communication between the free surface of the serous membranes and the lymphatic system, a communication which is more indirect than the former. This takes place by means of connective-tissue corpuscles, of which the processes reach upwards to the free surface, and in the opposite direction are in continuity with those of the deeper connective-tissue corpuscles of the matrix. The lymph-canulicular system may be said to communicate freely with the surface.

To this structure I have applied the term Pseudostoma. It is met with in most serous membranes, and especially on the lymphangeal nodules and cords of the omentum, and the pleura mediastini. In the latter situations many of the germinating endothelial cells found, as already stated, on the surface of these lymphatic structures, are seen to bud directly from the pseudostomatous cells. This process plays a very important part in inflammation of the serous membranes, as will be shown hereafter.

Fig. 9.



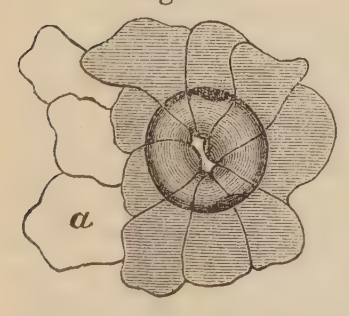
From a fresh preparation of oedematous pleura mediastini of cat, representing a lymphatic cord in profile.

a, Germinating endothelium of the free surface. *b*, Pseudostomatous cells. *c*, Matrix of the cord. *d*, Lymph corpuscles.—Magn. × 300.

7. *Development of Lymphatic Capillaries in the Serous Membranes.*

Lymphatic capillaries may be observed in development in the normal omentum of middle-sized rabbits, and in the mesogastrium of frogs. In both instances they develop by vacuolation of connective-tissue corpuscles, which are thus converted into vesicles (*see* Fig. 11). The wall of these vesicles is at a later stage differentiated into endothelial plates. In the frog these vesicles possess at an early stage of development a ciliated lining.

Fig. 10.



From a preparation of the pleural surface of centrum tendineum of guineapig.—Magn. × 160.

Showing a vacuolated cell forming a stoma by having opened itself towards the general surface.

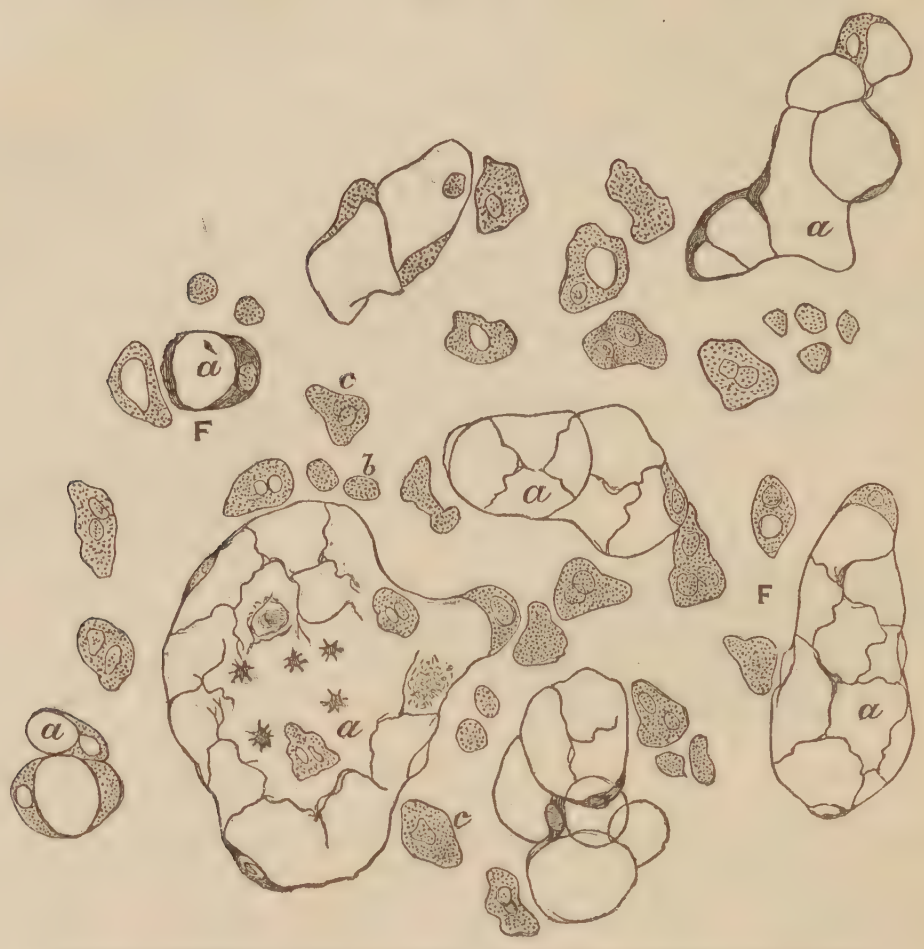
By the lengthening and eventual confluence of such endothelial vesicles true lymphatic tubes are formed, the wall of which consists of a single layer of endothelial plates. When a cell, representing a pseudostoma, is converted into an endothelial vesicle, which opens freely on the surface, a true stoma is formed (*see* Fig. 10).

8. *Development of Capillary Blood-vessels in the Serous Membranes.*

As has been already stated, in some serous membranes there is a constant growth and development of lymphatic nodules and cords, which after a certain period, possess a special system of capillary blood-vessels, the mode of development of which is as follows: The connective-tissue cells representing the stroma of these lymphatic structures are in direct continuity with the endothelial wall of existing capillary

vessels ; by the vacuolation of these cells, and by the eventual coalescence of the lumen of the vessel with the cavity of the vacuole, the capillary

Fig. 11.



From a preparation of the mesentery of a monkey suffering from chronic peritonitis.

a–a, Different stages of vacuolation of cells, in order to form lymphatic vessels. —Magn. × 240, *see* p. 11.

vessel enlarges, after which the protoplasmic wall of the vacuolated cells differentiates into endothelial plates.

B. *Pathological Conditions.*

1. *The Changes of the Superficial Endothelium.*—(*a*) When severe acute peritonitis is produced in a rabbit, guineapig, dog, or cat, by the introduction, *e.g.* of ammonia, of a solution of iodine in hydro-iodic acid, or of toxic pyæmic liquids (which last prove fatal in from 12 to 48 hours), into the peritoneal cavity, the mesentery, intestinal serosa, omentum, and the peritoneum of the centrum tendineum are seen to be extraordinarily hyperæmic, if examined during the first 24 or 48 hours. The abdominal cavity contains mostly, but not always, a greater or smaller quantity of sanguinolent fluid, with abundant separation of fibrin. In such cases, the endothelium of the surface is in great part loosened and detached, and the liquid contains an abundance of isolated membranous shreds composed of endothelial cells. Many of the endothelial cells, both those found free in the liquid and those still on the peritoneum, appear larger than usual, as if swollen, and their protoplasm is occupied by granules of various sizes. If the fresh membrane is examined microscopically, it may be seen that around those parts which have become deprived of their endothelium, the individual endothelial cells are distinctly granular, even in the surface view.

A further important fact is that the nuclei of the endothelial cells exhibit distinct appearances of division, and this sometimes very extensively. We not only find in the liquid a number of flat endothelial cells, in which the nucleus presents the most varied aspects of division, but in other places, such as the centrum tendineum and mesentery, we

see appearances quite different from those observed in the healthy condition ; thus, for example, on the endothelium, which covers the peritoneal side of that portion of the serosa which lies upon the tracts of tendons, and on which, as we have shown, germination is not observed in the normal condition, there is an active alteration of the nuclei over extensive spaces, from simple constriction to division into two or three small nuclei. In the case of the omentum it is more difficult to give a decided opinion as to alteration, for here extensive germination goes on in the normal condition.

Should, however, the inflammation be less intense, *e.g.* in the case of a peritonitis consequent on the entrance of air to the peritoneum, or of a peritonitis induced by the insertion of threads, or small solid bodies of starch (1–2 c.c. of a a tolerably concentrated mixture of starch and water), of Berlin blue (5–10 c.c. of 5 per cent. solution), of oil, or oil and starch—in such cases, we obtain an appearance somewhat different from the preceding. Here, indeed, there may be seen, after 24 or 48 hours, or later, isolated detached endothelial plates in the exudation, but the peritoneum exhibits only a few places where the endothelium is loosened or has disappeared. In silver-stained preparations the strongly coloured endothelial cells are seen to be granular and somewhat swollen in diameter, the nucleus has become more prominent, in short, they possess more the appearance of epithelium ; the nucleus of many of the cells is in process of division. On the omentum, and especially on the fenestrated portion, polyhedral endothelial cells whose nuclei are in process of division may be easily recognised. What is most worthy of observation is, that where germinating endothelium was observed in the normal condition a very active germination has now taken place. It extends over the abdominal surface of the centrum tendineum, as well as over the pseudo-stomata and stomata of the omentum and the mesentery. In some places the germination of the endothelium is of remarkable extent. Bud-like processes occur, which consist of young endothelial cells, and the cells so proliferating arrange themselves into cords which stretch freely over the surface. In the exudation we find a number of cells, which by their size, their granulation, and their nucleus, are sharply distinguished from the small pale blood or lymph-corpuscles. In consideration of what has been already stated, we may, I think, be allowed to regard the corpuscles, resembling endothelial cells, which occur in the exudation, as the products of the endothelium. Besides, there can be no doubt that a large number of small pale cells found in the exudation, originate by the division of the others, for on the warm stage, amœboid movements and division are observed in the large cells, which have a constricted or double nucleus.

There are intermediate stages between the forms first described and those seen in acute peritonitis of more moderate degree, just as there is a complete gradation in acute peritonitis itself between the severest degree and the mildest ; and this depends on a variety of circumstances, such as the intensity and extent of the irritation, the kind of animal observed, and other particulars unnecessary to detail in this place. What we wish to show is the difference in respect to changes of the endothelium between a very acute peritonitis, with intense hyperæmia, abundant emigration of colourless blood corpuscles, and abundant thin exudation fluid, and slight acute peritonitis, with slight hyperæmia, generally limited to the omentum, peritoneum of the centrum tendineum and intestinal covering, and no marked emigration of colourless blood corpuscles. For in the latter case, an active germination of the endothelial cells takes place, limited chiefly to those parts where germination could be made out in the normal condition, namely, on the centrum tendineum around the stomata over the lymphatic capillaries of the

peritoneal surface, on the lymphangeal knots and cords of the omentum and of the mesentery (where we found pseudo-stomata and true stomata in numbers), and lastly, on the fenestrated parts of the omentum, where isolated pseudo-stomata are seen in the normal state.

(*b*) *Chronic Peritonitis.*—The condition of limited endothelial germination is still better observed in chronic inflammation. If we tuberculise* a guineapig by the well-known method of injecting material of an indurated lymphatic gland into the abdominal cavity, germination of the endothelium is seen round the stomata, especially on the omentum, parietal peritoneum, centrum tendineum, and mesentery. The process is most intense on the omentum. Here we find, so long as the tuberculous process has not advanced extensively, active germination of the endothelium on the knots and cords already so frequently mentioned, the number and size of which have decidedly increased. Under these circumstances the true stomata can be made out in many cases much more easily than in the normal condition, both in fresh preparations and those treated with silver. Their number, compared with that of the pseudo-stomata, is decidedly smaller.

The germination of young endothelium on the fenestrated portions in the form of small buds is also very active. The centre of each little bud is represented by a protoplasmic central stalk emerging from the matrix, which corresponds in structure to a pseudo-stoma.

As has been already indicated, the germination of the endothelium

Fig. 12.

Peritoneal surface of the centrum tendineum of guineapig suffering from artificial tuberculosis.

a, The straight lymphatics. *b*, Stomata surrounded by germinating endothelium *c*.—Magn. × 300.

* For a detailed account of the macroscopical conditions of the affected organs, see Eleventh Report of the Medical Officer of the Privy Council.

surrounding the true stomata is also active. Not only do the endothelial cells enlarge, become distinctly granular, present nuclei in the act of division, and germinate so as to produce lymphoid cells, but this change gradually spreads to the endothelial cells of the neighbourhood, as well as to those of the vertical lymph-canals, and to those of the lymphatic capillaries into which these lymph-canals open. If we examine the abdominal surface of a diaphragm furnished with abundant nodules of tubercle, we recognise the most varied stages of development in the germination of the endothelium of the stomata, from small buds to large patches and nodules composed of polyhedral endothelial cells. In chronic inflammation there is also abundant germination of the endothelium of true stomata on the omentum.

A fact worth noticing is, that guineapigs in which tuberculosis has been induced by subcutaneous injection, or by direct injection into the blood-vessels, exhibit a marked difference from those cases in which it is induced by injection into the peritoneum. Here the tubercular process being more advanced in the peritoneum than in the internal organs (the lymphatic glands, spleen, liver, and lungs), the germination of the endothelium round the stomata or pseudostomata is the first and chief change to be observed when the tubercular process has made little progress. This is not the case when the tuberculosis has been induced subcutaneously or by the vessels, for then no marked growth of endothelium round the stomata takes place until the tuberculosis in general is very advanced.

What has been described as regards the omentum and centrum tendineum holds good of the mesentery. Where it exhibits moderately advanced tuberculous affection, the growth of the endothelium around the true stomata occurs in a very striking manner.

Precisely the same facts may be seen in the rabbit. If a few c.c. of starch and oil or starch and water are injected into the abdominal cavity of a well-nourished rabbit, and the omentum and centrum tendineum are examined four or eight weeks later, we find that the alteration of the endothelium is limited to those places where we have previously seen stomata and pseudo-stomata, the germination of endothelium being here very considerable.

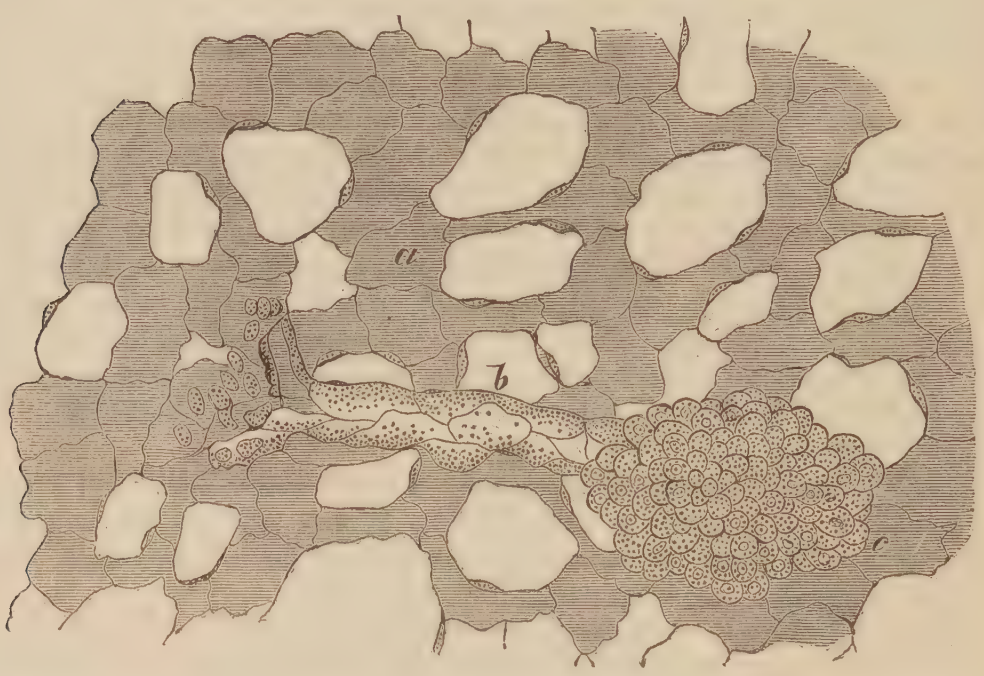
Germination of the endothelium round the stomata of the mesentery and mesogastrium is frequently observed to take place in female frogs during the winter months. In these animals the septum of the cisterna lymphatica magna shows abundant germination round the stomata on the peritoneal surface, as well as of the adjoining endothelium. It may be remarked by the way that the peritoneum of the ovaries and oviducts, and of the neighbourhood of the liver, were more or less covered with false membranes.

2.—*Alteration of the Cellular Elements of the Matrix of the Serous Membranes.*—(*a*) *Acute inflammation.*—In acute inflammations of the serous membrane, produced by injecting irritating substances, such as diluted ammonia, starch, and especially certain pyæmic and septicæmic exudation liquids, into the peritoneal cavity, the omentum and mesentery often exhibit various degrees of œdema. In such œdematous membranes the lymph-canalicular system and lymphatic vessels are seen to be much dilated by plasma, and the connective-tissue corpuscles lining the lymph-canalicular system are easily recognised even in the freshly prepared membrane as swollen, granular, flattened, branched cells. When a serous membrane, *e.g.* the peritoneum of the centrum tendineum, or the omentum, has been inflamed for 48 hours, some striking changes may be observed. In the lymph-canalicular system the lacunæ are seen to be much larger and less branched than in the normal condition, the

canals are wider, and a few migratory cells are found; the connective-tissue corpuscles are more granular and their nuclei constricted. In some places a still greater alteration may be seen, where the connective-tissue cells of the lymph canalicular system have undergone complete division.

(*b*) *Chronic inflammation.*—In chronic inflammation these changes are well marked. In guineapigs, in which artificial tuberculosis has been induced, the alteration of the connective cells in the matrix of the

Fig. 13.



Silver preparation of the omentum of guineapig suffering from artificial tuberculosis.
a, Trabeculæ of fenestrated omentum. *b*, A freely projecting villus.
c, Its top covered with germinating endothelium.—Magn. × 300, *see* p. 17.

serous membranes is well seen. The connective-tissue corpuscles lining the lymph-canalicular system appear to have undergone very rapid

Fig. 14.

From the mesentery of a monkey suffering from chronic peritonitis, representing a morbid projection.

a, Its peduncle. *b*, Its top covered with germinating endo lium.
A vacuolated cell of its matrix. *d* Lymphoid corpuscle.—Magn. × 400, *s e* p. 1 .

division ; they are in many places crowded together and less branched, and exhibit the aspect of an endothelium. By such germination regular patches and nodules may be formed. As might be expected, all the lymphangeal structures we have previously mentioned increase in size by the germination of the connective-tissue cells constituting their matrix ; and such structures are formed in chronic inflammation in the same way as they are formed in the normal condition, but in some cases to an enormous extent. In some animals the omentum, the *peritoneum parietale* and the mesentery are seen to be covered with papillary or villous projections, which are sometimes attached to a large lymphangeal cord or nodule of the matrix, but sometimes spring from a thin part of the membrane. These papillæ or villi are generally covered in some places with germinating endothelium, and are either a single outgrowth of a pre-existing lymphangeal nodule or cord, or originate from a pseudo-stomatous cell, which has undergone rapid germination, and has carried with it the surrounding zone of endothelial cells as a covering (*see* Figs. 13 and 14).

3.—*The Blood Vessels and Lymphatic Vessels.*—In chronic inflammation there is a very extensive new formation of blood capillaries and lymphatic capillaries. This occurs in consequence of the newly-formed lymphangeal nodules becoming supplied with their special vessels.

The mode in which capillary blood-vessels and lymphatics are formed in chronically inflamed serous membranes is precisely the same as in the normal condition.

Fig. 15.

From the fenestrated portion of an injected omentum of a guinea-pig suffering from slight artificial tuberculosis.

a, Arteries. *b*, Veins. *c*, Capillary blood vessels. *d*, Fenestrated membrane. *e*, Freely projecting lymphatic nodule.

Finally, it may be stated that there is in some cases an extensive formation of endolymphangeal nodules in pre-existing lymphatic vessels.

App. No. 3.

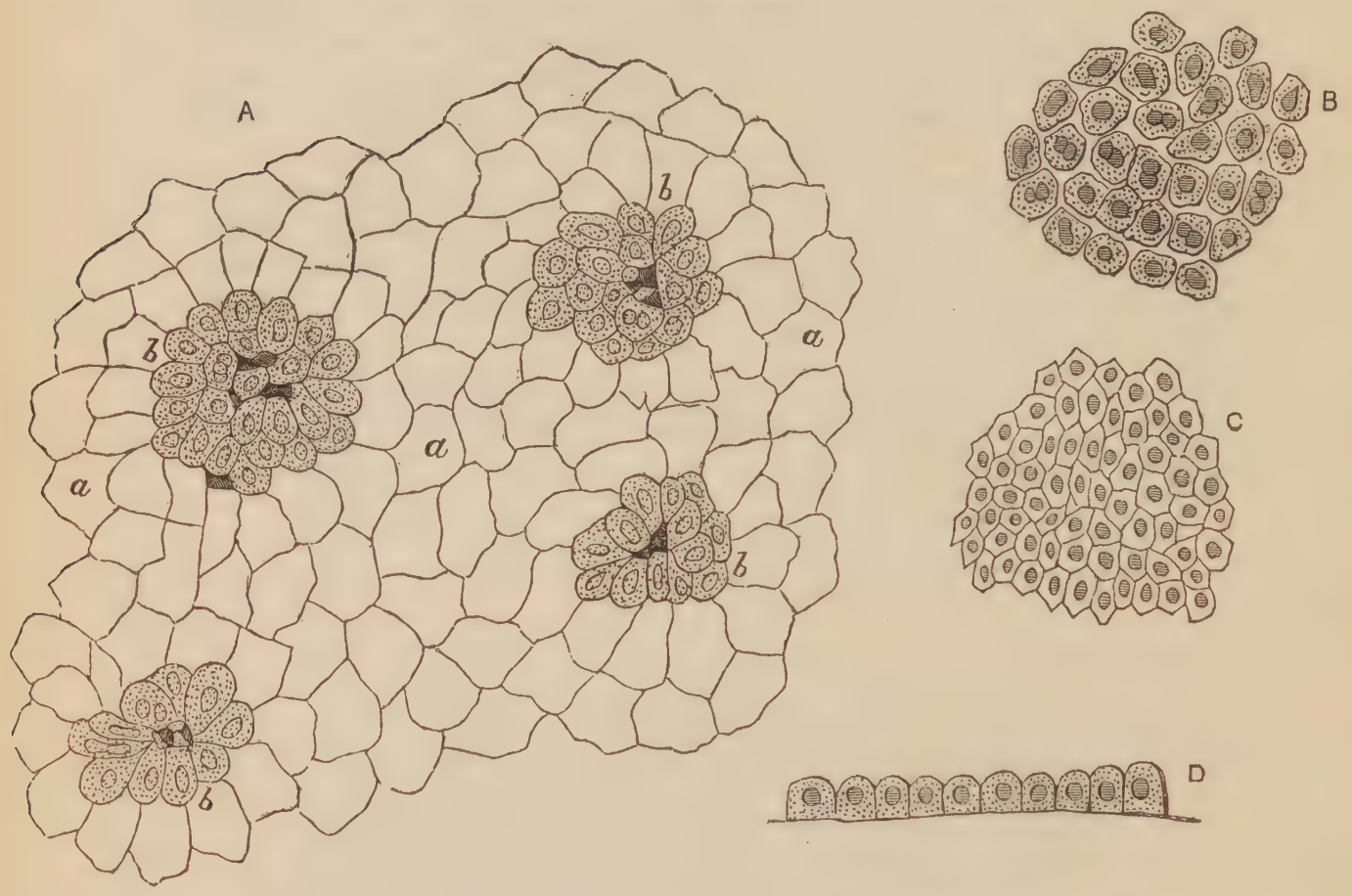
On the Lymphatic System and its relation to Tubercle, by Dr. Klein.

Part II.—Lymphatic System of the Lungs.

A. *Normal Anatomy.*

The endothelium of the surface of the lungs consists, in the normal condition, of polyhedral cells (not flattened as commonly described) arranged in a single layer. This is well seen in guineapigs, less distinctly in rabbits, rats, dogs, and cats. If the lung is not distended, the endothelium of the surface very much resembles an epithelium, the cells being polyhedral, or in the form of short columns; they are

Fig. I.



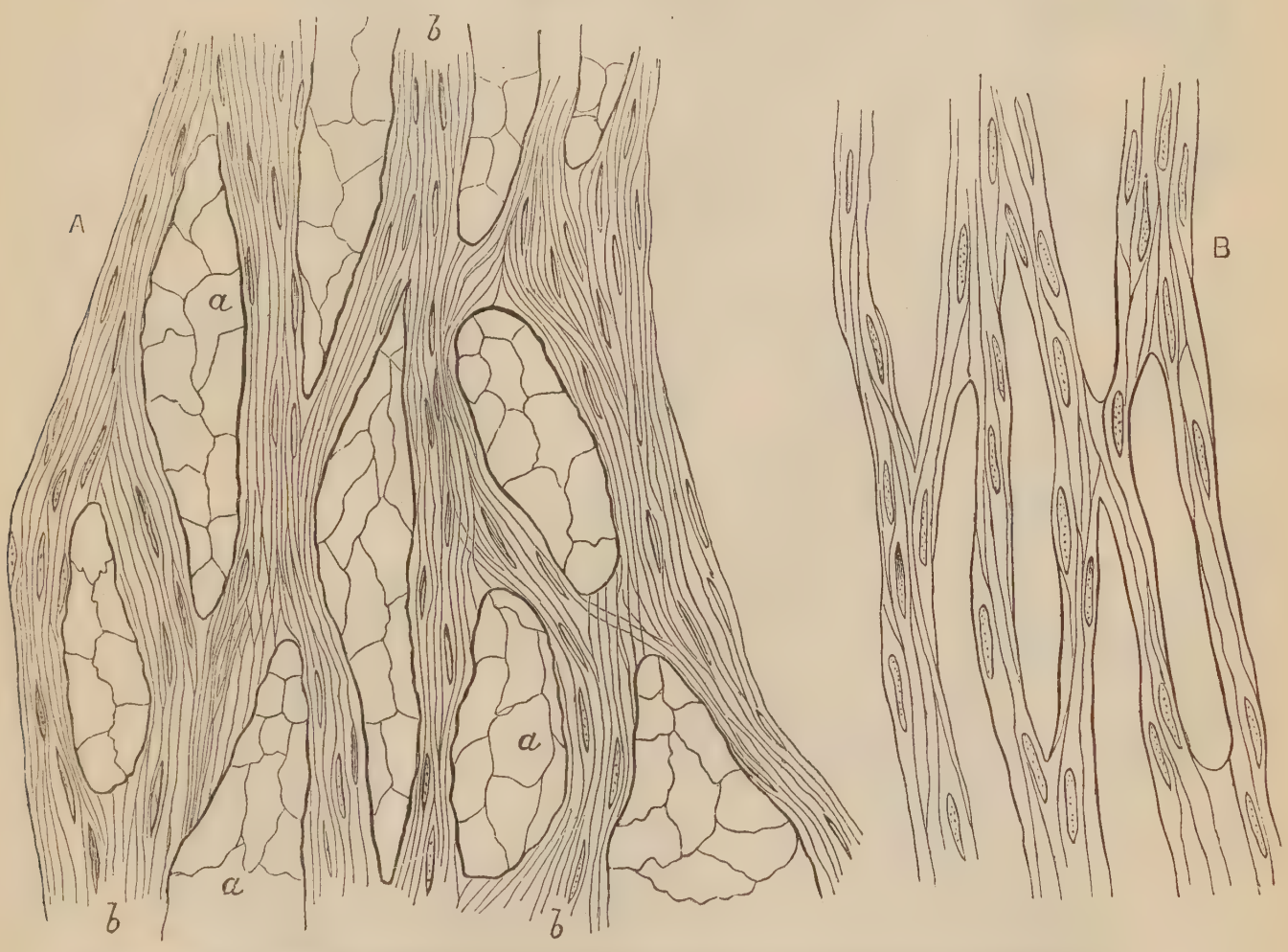
A, Surface view of the endothelium of the pulmonary pleura of a rabbit in the condition of chronic pleuritis, *see* p. 21.
a, General flattened endothelium. *b*, Germinating endothelium around stomata.
B, Endothelium of the pulmonary pleura of a guineapig suffering from artificial tuberculosis, the endothelium being viewed from above.
C, Endothelium of a normal pulmonary pleura of guineapig, viewed from above.
D, The same endothelium viewed in profile.—Magn. power, 300.

markedly granular, and have distinct nuclei. Even in the moderately distended lung, the endothelium of the pleura pulmonum is by no means of the same morphological character as that on the costal pleura. Between the endothelium of the one and that of the other organ there exists the same difference as between that of the ovary and that of the peritoneum—the one consisting of polyhedral, or shortly columnar, granular cells with very marked nuclei, the other of very flattened, almost hyaline, endothelial plates.

The pleura pulmonum is a very thin connective-tissue membrane, provided, like other serous membranes, with a rich network of elastic fibres. In the lungs of the rat, rabbit, cat, and dog the pleura pulmonum seems to consist, for the most part, of elastic networks. In the matrix, there is generally one layer of flattened connective-tissue corpuscles to be found.

Beneath the proper pleural membrane there exists, in the guineapig, a membrane which consists of non-striated muscular fibres, arranged in bundles which form a meshwork. In the normal condition the bundles are relatively thin, and the meshwork which they form has elongated large meshes. In the distended lung the meshes are of a much greater diameter than in the collapsed lung; in the latter they form a more continuous membrane. The muscular bundles have, in general, a radiating direction from the apex towards the basis of the lung; and it is further to be noted that they are most abundant on the external surface, viz., that directed towards the anterior wall of the chest, and the internal surface, viz., that directed towards the mediastinum; whereas on the posterior surface the bundles are scanty, and become more and more so the nearer the vertebral column is approached. This distribution of the muscular tissue is therefore in perfect agreement with the proportion in which the different parts of the lung participate in the respiratory movement, the fibres being most richly distributed over those parts of the pulmonary surface which are subject to the greatest extent of excursions, and *vice versâ*. In rats and rabbits, as well as in cats and dogs, bundles of unstriped muscular fibres occur sparingly; at any rate there are none on the posterior surface of the lung of these animals. As soon as the superficial parts of the lung

Fig. II.



A, Meshwork of unstriped muscles in the pulmonary pleura of a guineapig suffering from artificial tuberculosis:
a, Intermuscular lymph spaces lined by endothelium. *b*, Muscular trabeculæ.—Magn. × 300.
B, Meshwork of unstriped muscles in the normal pulmonary pleura of guineapig.—Magn. × 300.

become the seat of a chronic inflammatory process (*e.g.* tuberculosis, chronic pneumonia), the muscular bundles increase in breadth and number to such a degree, that they form a continuous membrane, chiefly in those parts of the surface which correspond to the diseased portions of the lung.

FIG. III.

Injected subpleural lymphatic vessels of a guineapig's lung.

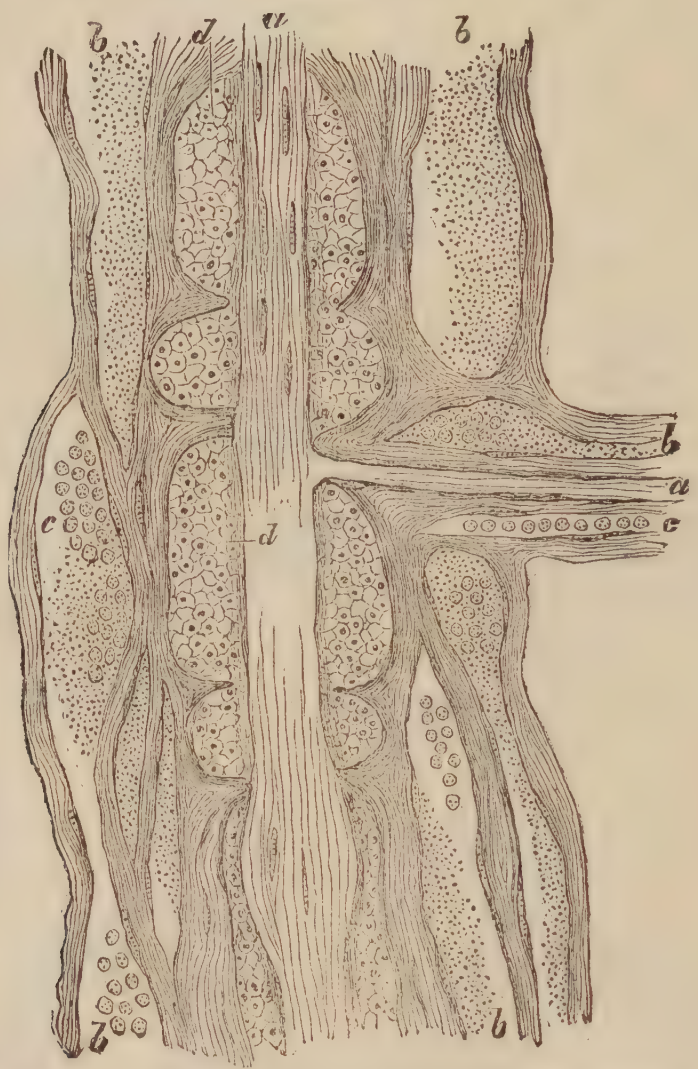
a, Main branches provided with valves. *b*, Branches emerging from the depth, *i.e.*, the interalveolar tissue.—Magn. × 90. *See* p. 21.

1. *Subpleural lymphatics.*—The meshes of the muscular membrane of the lung of guineapigs are lined by a single layer of flattened endothelial cells, constituting, in fact, a communicating system of lymphatic sinuses. I call this system of lymphatics the *intermuscular or pleural lymphatics.* In the distended lung of the guineapig, these pleural lympathic sinuses are seen to be covered by hardly anything but the endothelium of the pleural cavity, between which and the cavities of those sinuses a free communication exists by means of true stomata; so that the endothelium lining the sinuses is here directly continuous with that of the pleural surface. In every case of chronic pleuritis induced by injecting irritating substances (such as products of acute and chronic pyæmic processes, products of indurated lymphatic glands), an active germination of the endothelium around those stomata takes place (*see* Fig. I.). This germination extends not only to the endothelium of the neighbouring parts of the pleural surface, but also to the endothelium of the intermuscular lymphatic sinuses. The relation between the cells of the membrana propria of the pleura pulmonum and the endothelium of the surface, is similar to that already described by me in other serous membranes, the cells of the *propria* throwing out processes, which project between the endothelial elements of the free surface, thus forming pseudostomata. The pleural lymphatics stand in communication with lymphatic tubes, which lie in grooves, the arrangement of which corresponds with that of the most superficial groups of alveoli of the lung. These may be called the *subpleural lymphatics;* they are provided with valves, and form a network of anastomosing lymphatic vessels. The larger trunks run along the ligamenta pulmonum towards the root of the lung. This system of lympathic vessels is best developed in the lung of the dog, in which it has been described by Wywodzoff; it is also well developed in the lungs of rabbits and cats. It receives lymphatic branches, which take their origin between the alveoli of the superficial portions of the lung. The mode of origin of these interalveolar lymphatics is that already described in my report on the serous membranes. The septa of the alveoli contain branched connective-tissue corpuscles; the spaces in which these cells lie, forming the lymphcanalicular system, open into the cavities of the interalveolar lymphatics, with the endothelium of which the cells of the lymphcanalicular system are in direct continuity.

2. *Perivascular lymphatics.*—Besides the system of subpleural lymphatics, the lung contains two other systems; of which one takes its origin in the alveolar septa from branched cells exactly like those previously referred to. The lymphatic capillaries of this system lead into vessels that accompany the branches of the pulmonary artery and vein; they run either in the adventitia of these vessels in twos or threes, anastomosing with each other, or the blood-vessel is entirely, or only half invaginated in a lymphatic vessel (*see* Fig. IV.). The branched cells of the alveolar septa, from which the capillaries of this system of lymphatics (which we will call the *perivascular lymphatics*) originate, have an important relation to the epithelium of the alveoli; for they send a process, or a greater or less portion of their body, between the epithelial cells into the cavities of the alveoli. These represent pseudostomata, such as I have described for the serous membranes. As these branched cells have a corresponding lymphcanalicular system, it is easy to understand why Sikorski, in his experiments, found that carmine entered freely from the cavities of the alveoli into the interalveolar lymphatics. But there is no other communication between the cavities of the alveoli and the lymphatics than by means of these pseudostomata. It can be easily understood that the pseudostomatous canals (viz. the canal in which lies the process of a cell

projecting freely into the cavity of an alveolus, and the lymphcanalicular system, in which the interalveolar branched cells lie) may become occasionally distended, *e.g.* in inflammation, by exudation, or by migratory

Fig. IV.



From a vertical section through the lung of a guinea-pig suffering slightly from artificial tuberculosis, *see* p. 21.

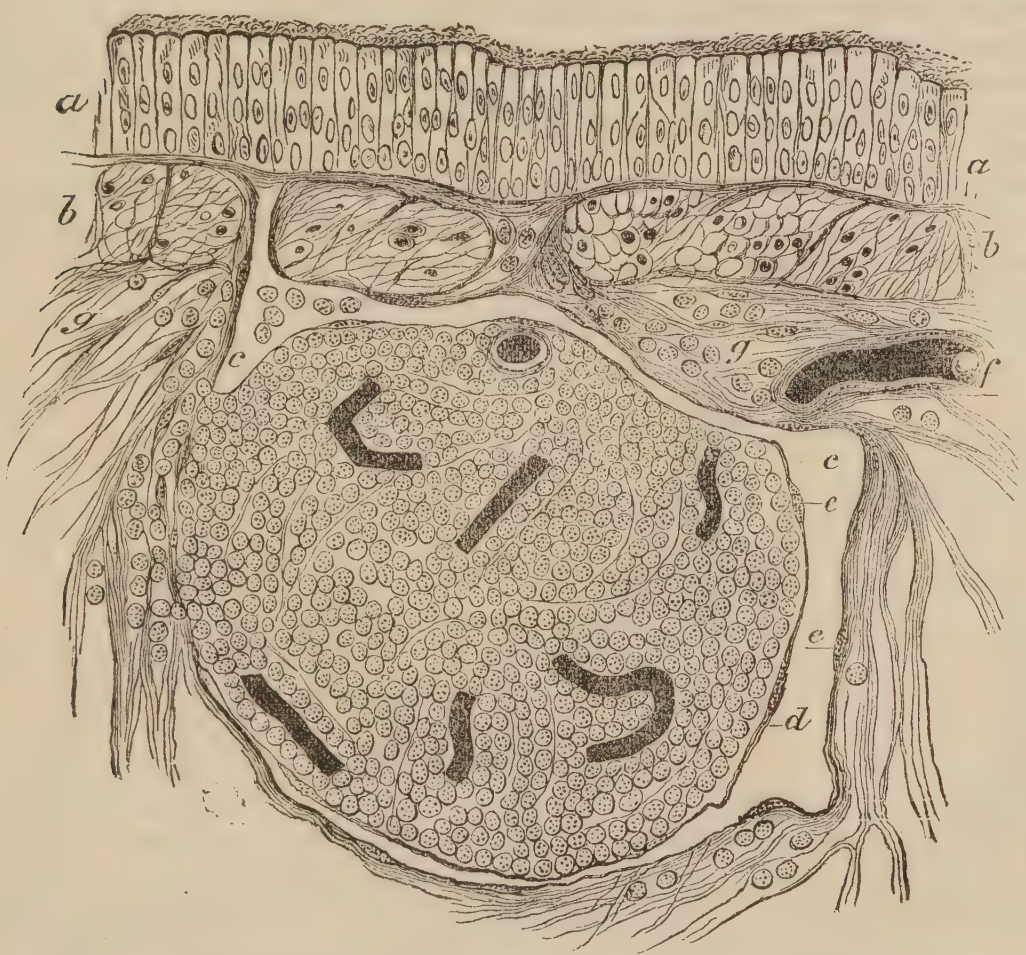
a, Branch of pulmonary artery. *b*. Perivascular lymph-spaces, containing granular material and lymph-corpuscles *c*. *d*, Transversely cut muscular bundles.—Magn. × 160.

cells. In fact, it must be assumed that cells, such as are produced by catarrhal inflammation of the air-passages, migrate from the cavities of the alveoli into the interalveolar lymphcanalicular system through those pseudostomata ; and the same assumption must be made for the well-known large granular mucous corpuscles, which in many lungs contain carbon particles, inasmuch as similar cells are found in the interalveolar tissue.

3. *Peribronchial lymphatics.*—The third system of lymphatics is composed of lymphatic vessels which are chiefly distributed in the adventitia of the bronchi. I shall therefore call it the system of *peribronchial lymphatics.* The vessels of this system are usually distributed around the bronchi, anastomosing with each other, and especially with the perivascular lymphatics. The vessels of the peribronchial system take up capillaries, which originate in the mucous membrane of the bronchi and penetrate through the tunica muscularis of the bronchi. These capillary branches originate in the usual way ; *i.e.* their wall is continuous with the branched cells of the mucosa, which cells in turn penetrate, as a

nucleated reticulum, between the epithelial cells of the bronchus, and project on its free surface. From this it may be understood how particles can penetrate from the cavity of a bronchus into the peribronchial lymphatics, as in the experiments of Sikorski. The lymphatics are always most numerous on that side of a bronchus which is directed towards a branch of the pulmonary artery. In the course of each bronchus, especially those that possess only a thin muscular tunic and no trace of cartilage, there are generally several vascular lymph-follicles to be met with, which are placed in continuity with the endothelial wall of a lymphatic vessel, in such a manner that they are surrounded by that

Fig. V.



From a longitudinal section through the wall of a bronchus of a guineapig's lung.

a, Ciliated epithelium. *b*, Muscular coat. *c*, Lymph-sinus surrounding an injected lymphatic follicle *d*. *e*, Its endothelium. *f*, Injected blood vessels. *g* Lymph corpuscles. —Magn. × 300.

lymphatic vessel, in the same way as the lymph-follicles of Peyer's patches are by their lymph-sinuses. These follicles, already seen by Dr. Burdon Sanderson, extend up to the tunica muscularis ; in some instances they are to be traced through this latter into the mucosa. They always lie in the wall of a lymphatic vessel, between the bronchus and the accompanying branch of the pulmonary artery. They are of different sizes, and are generally spherical or elliptical ; sometimes they represent merely a cord-like thickening of the wall of the lymphatic vessel. In the lung of the guineapig these peri-lymphangeal follicles are very numerous ; they are not so numerous in rabbits. It can be proved that a constant growth and reproduction of these follicles is going on. The lymphatic vessels of the two last-mentioned systems, anastomose with each other in the ligaments of the lung, and finally enter the bronchial lymphatic glands.

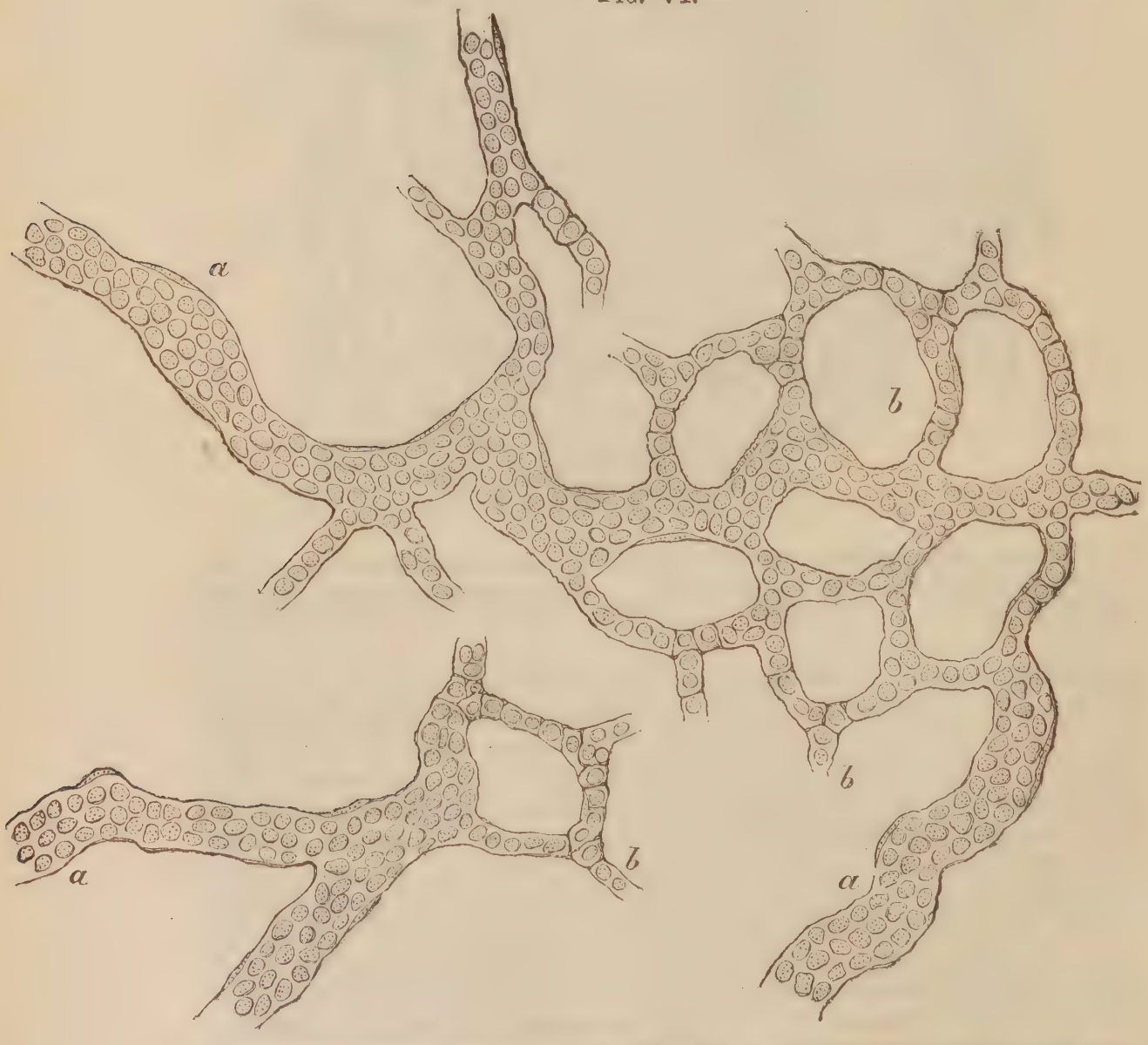
B. *Pathological Conditions.*

I have already mentioned the germination of the endothelium of the surface, and the hypertrophy of the muscles, in chronic diseases of the lung.

In many chronic inflammatory processes of the lung (chronic pyæmia, artificial tuberculosis, chronic pneumonia) the pleura pulmonum becomes the seat of nodules of various sizes and shapes. Generally they are more or less round, and correspond in position to those superficial portions of the lung which have become the seat of an inflammatory process. These nodules of the pleura are due to a very rapid germination of the branched connective-tissue corpuscles, simultaneously with an increase of fibrous connective-tissue, this latter fact being very obvious when the nodules have reached a certain age. As long as they are small, they show merely an abundance of cellular elements; in their later stages they become richly supplied with capillary blood-vessels.

Lungs of guineapigs that are far advanced in the process of artificial tuberculosis (*i.e.* where the bronchial glands have already become the seat of cheesy deposits) show superficial nodules, which are in direct

Fig. VI.



Subpleural lymphatics of a guineapig suffering from artificial tuberculosis, *see* p. 25.

a, Main branches. *b*, Interalveolar branches; both are filled with lymph-corpuscles; in some places the lymphatics are in the act of becoming converted into cords of adenoid tissue.—plagn. × 300.

continuity with the subpleural lymphatics. In horizontal sections through such portions of the lung, one finds these lymphatics filled with lymph-corpuscles, while at a later period they are occupied by an adenoid reticulum, the meshes of which contain lymph-corpuscles, and which is in direct continuity with the endothelium of the lymphatic tubes (*see* Fig. VI.). The nodules themselves represent a network of cords, which very much resembles adenoid tissue. The meshes of this network of trabeculæ are the alveoli, which, at an early period, contain a few lymphoid corpuscles, while the epithelium is, at the same time, in a state of germination, the individual cells being swollen and the nucleus in a state of division. At a later period the alveoli are filled with small lymphoid corpuscles, while the epithelium of the alveoli is no longer to be distinguished as such. The blood-capillaries belonging to these alveoli have undergone some remarkable changes, of which I shall speak at length afterwards; at present I will only mention that at a later period they are no longer permeable for the blood. These interalveolar trabeculæ of adenoid tissue, forming the framework of the superficial nodules, are developed from the branched connective-tissue corpuscles of the alveolar septa. The same process extends to the subpleural lymphatics, originating from these interalveolar connective-tissue corpuscles, in such a way that these lymphatics become converted into cords of adenoid tissue connected with their endothelium. Consequently these lymphatics become converted into endo-lymphangeal cords.

In the same stage of the process of artificial tuberculosis characterized as above stated by the presence of cheesy deposits in the bronchial glands, two kinds of morbid structure can be distinguished by the naked eye on the surface of the lungs :—

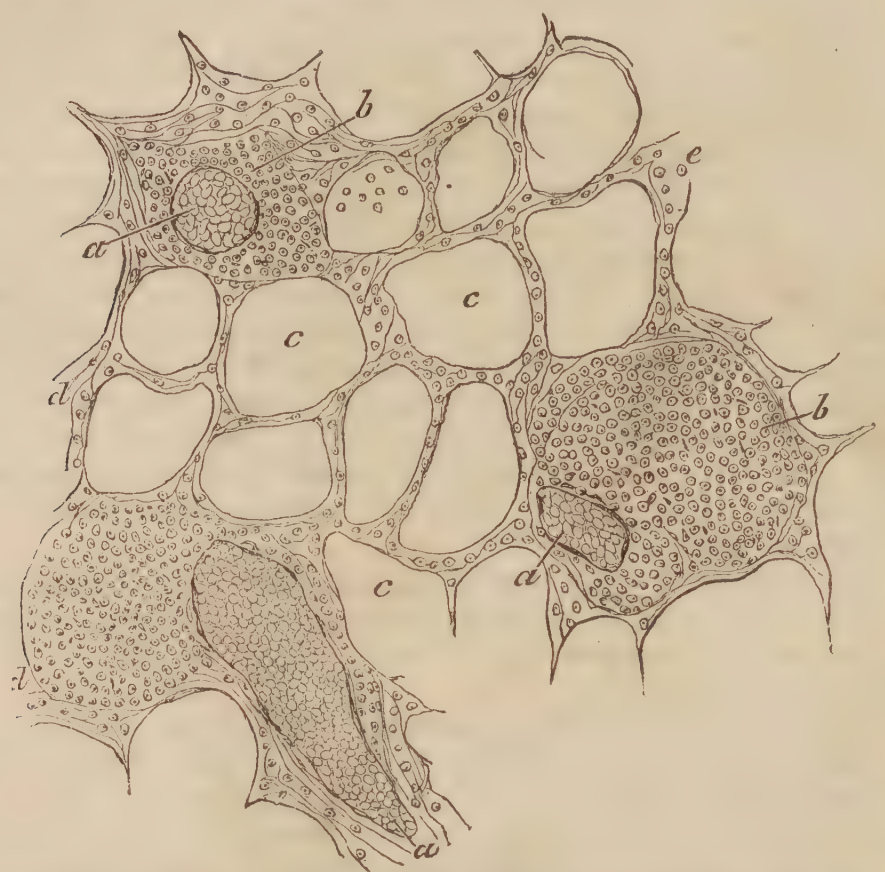
(*a*) Translucent structures of a circular or irregular shape, sometimes projecting slightly above the surface, generally isolated, but in some instances confluent, so as to form patches. The smallest are of the size of the head of a small pin ; some of them are three, four, or several times as large. In some lungs only the large structures are to be found; the larger kind have generally a yellowish centre.

(*b*) Opaque patches of considerable diameter projecting above the surface of the lung, some of them relatively very large (about $\frac{1}{6}$ to $\frac{1}{10}$ of an inch), quite white, and very firm. On sections through the lung one finds that the first kind of structures correspond with cords provided with lateral nodular swellings, which accompany the branches of the pulmonary artery and vein. The second kind of structures correspond with nodules and patches which are irregularly distributed in the tissue of the lung. On microscopical examination it is seen that the first kind of structures are perivascular cords of adenoid tissue, representing the follicular tissue which is found in the walls of the peribronchial lymphatics in the normal condition (*see* Fig. VII.). Many of these perivascular cords or nodules are supplied with a system of capillary blood vessels. The second kind of nodules, or patches, are seen to consist, on microscopical examination, of a framework of trabeculæ which corresponds to the interalveolar tissue ; they represent trabeculæ of adenoid tissue which are in continuity with the perivascular cords first mentioned. The meshes of this network are more or less filled by cells lying in the spaces that were previously the cavities of the alveoli. The question arises, How do these two kinds of morbid structures develop, and what is their ultimate fate ?

If one studies sections of lungs that possess very few of the first kind of cords and nodules, one comes across a number of the lymphatic vessels that accompany the branches of the pulmonary artery, containing more or less numerous lymph-corpuscles. In addition to those

just mentioned, lymphatic vessels may be found the endothelium of which is in continuity with a thin short cord of adenoid tissue that stretches along the outer wall of the lymphatic, or (as may be seen in some places) projects into its cavity, thus connecting the two endothelial walls of the lymphatic ; in other words, we have here a peri- as well as an endo-lymphangeal growth of adenoid tissue, connected with the

Fig. VII.



From a vertical section through the lung of a guineapig suffering from artificial tuberculosis. *a*, Blood vessels in transverse section filled with blood. *b*, Perivascular cords of adenoid tissue. *d*, Nodular swelling of the latter. *c* Alveoli. *e*, Interalveolar tissue.—Magn. × 60, *see* p. 25.

endothelium of the lymphatic. From what I have shown in the case of the serous membranes, there can be little doubt that the above-mentioned tuberculous cords of adenoid tissue accompanying the blood-vessels are in reality only peri- or endo-lymphangeal outgrowths of the endothelium of the lymphatics. It is important to state that, at the same time, the follicles of the bronchial adventitia increase in size, and also that a peri-lymphangeal new growth takes place on the peribronchial lymphatics. From the study of the normal lung, it can be ascertained that all the large branches of blood-vessels are not accompanied by lymphatics, nor even a single branch along its whole length, but that in some places they are only surrounded by branched connective-tissue corpuscles, which may be said to belong to their adventitia. In a particular case, it cannot be determined whether a certain tubercular cord has developed by the increase of these adventitial cells, or whether it has developed from the endothelium of a lymphatic, either as a peri- or endo-lymphangeal cord ; for the fully developed cords have the same relation to the blood-vessels as if they had developed in their adventitia.

I have already mentioned that the growth of adenoid tissue in the branches of the subpleural lymphatics extends to the connective-tissue corpuscles between the alveoli. Exactly in the same way we see the perivascular adenoid cords spreading between the alveoli; that is to

say, the perilymphangeal growth of tracts of adenoid tissue extends from the lymphatics to the interalveolar branched cells, with which the endothelium of the former is in direct continuity.

The first points at which the tubercular perivascular cords of adenoid tissue make their appearance are the ultimate branches of the pulmonary artery and vein, whence they spread along the lymphatics towards the

Fig. VIII.

From a section through the lung of a tuberculous guineapig.

a, Branch of pulmonary artery. *b*, Branch of pulmonary vein. *c*, Tubercular nodule. *d*, Tubercular cords.—Magn. × 60.

larger branches as well as towards the interalveolar branched cells. In general the growth in the first direction (that is, towards the larger branches) goes on much more abundantly and rapidly than in the other direction.

The following important facts are constantly met with in the tuberculous lungs of guineapigs:—The ultimate branches of the pulmonary artery show a germination of their endothelium, which is already recognizable in the earlier stages of the disease, at a time when perivascular cords are only rarely to be found. If the process advances, the germination of that endothelium reaches such a degree that the cavities of the blood-vessels are almost filled with its products, only a very narrow central canal being left free. In later stages, the tunica media of the smaller and middle-sized vessels, that are provided with perivascular cords, becomes very much thickened, and splits into laminæ,

between which lie accumulated lymphoid cells, either free or contained in a reticulum. In many places it can be shown that the adenoid tissue of the perivascular cords gradually grows towards the cavities of the vessels, and finally assumes the whole portion of the vessel into its substance. The chief fact of importance, however, is that the capillary blood-vessels of those interalveolar trabeculæ, into which the perivascular cords have penetrated, have become converted into solid nucleated bands and threads, which are in continuity with the surrounding reticulum. These threads, although they appear solid, must be taken as still permeable by fluid substances; for in lungs, the pulmonary artery of which had been previously injected with a cold solution of Berlin blue, the cavity of many of the capillaries in the neighbourhood of these interalveolar trabeculæ stops short, but the injecting material can be traced into the nucleated filaments which enter these trabeculæ. From the study of a great number of specimens taken from lungs in different stages of the process of artificial tuberculosis, I have reason to believe that the first parts which undergo inflammatory changes are the ultimate branches of the pulmonary artery and the capillaries next to them, and that the morbid process extends from them to the corresponding lymphatics.

I have already mentioned that where the alveolar septa become

Fig. IX.

From the same lung as Fig. VIII.

Branch of pulmonary artery. *b*, Capillary blood vessels surrounding the alveoli. *c*, Perivascular adenoid tissue. *d*, Epithelium of alveoli. *e*, Giant-cells, having originated by the fusion of several epithelial cells.—Magn. × 400, *see* p. 29.

thickened, the epithelium of the alveoli becomes gradually changed, so as completely to fill the cavities of the alveoli. By this means nodular or patch-like structures are formed, which may be called secondary patches. It may be said, in general, that the epithelial cells germinate: they enlarge; their nuclei divide; and then the cells themselves divide. In many alveoli there appear, besides isolated epithelial cells, with or without carbon particles in their substance, numerous small lymphoid corpuscles. In some of the alveoli the enlarged epithelial cells become fused together to one large mass of granular protoplasm, which contains a number of nuclei in its periphery; this represents, in the true sense of the term, a "giant cell" (*see* Fig. IX.). We may therefore say that, at an early period, these patches consist of trabeculæ, which represent the thickened interalveolar septa and their meshes (the alveoli), and that the latter are filled either with small cells or with giant cells, or rather with multinuclear protoplasmic cylindrical masses. These secondary patches gradually increase in size, by the extension of the adenoid metamorphosis of the alveolar septa, and the changes of the capillary blood-vessels, indicated above.

A perivascular cord may become furnished with a number of lateral nodules of adenoid tissue from the assumption, by adenoid interalveolar cords, of the contents of alveolar cavities into their own tissue. Where, however, the alveolar cavities contain giant cells, other remarkable changes take place. These are as follows:—The cylinders of multinuclear protoplasm grow and divide into a number of giant cells, which gradually become converted into a tissue to a certain extent resembling adenoid tissue, but differing from it in many respects. Thus the giant cells give origin to a more or less regular network of nucleated cells, which, consisting at first of granular substance, soon assumes the appearance of a more or less distinct fibrillar substance; in their meshes lie only a limited number of lymphoid cells. This tissue spreads very rapidly, and finally undergoes, from the centre outwards, a fibrous degeneration, which becomes the seat of cheesy deposits.

Different lungs are somewhat different in this latter respect. In some cases the transformation of the giant cells into a network of nucleated cells goes on very rapidly; and then the cheesy metamorphosis is also soon established. In other cases the growth of the network of nucleated cells has a very long duration, and consequently the growth of the secondary patches remains active for a long time. The network of nucleated cells is, at no period of its development, such a delicate reticulum as in the adenoid tissue, nor does it contain lymphoid corpuscles so constantly as this latter. Moreover the adenoid tissue of the perivascular cords or their lateral nodules *never becomes the seat of a fibrous or cheesy metamorphosis.* The more the lung has advanced in the process of artificial tuberculosis, the more do we find the tissue of the lung, in the neighbourhood of the primary and secondary nodules, undergoing inflammatory changes which consist in thickening of the alveolar septa, and in a granular condition of the walls of their capillary blood-vessels, the nuclei of which are in active germintion, their number being disproportionately large.

In the peripheral parts of the lung the most numerous secondary nodules are to be met with; and consequently the most numerous cheesy deposits are here to be found. I have often seen a system of large patches projecting somewhat above the surface and radiating towards the deeper parts, as the terminal branches of a minute bronchus pass towards the stem.

The secondary process extends from the terminal branches (alveoli and infundibula) to the large bronchi. In these the process becomes very marked, and consists of the following changes:—

(*a*) The epithelium germinates very abundantly, whereby the cavity may finally become almost completely plugged up by the progeny of the epithelium.

(*b*) A more important change consists in the germination of the tissue that we have designated above as pseudostomata, namely, the branched cells of the tunica mucosa that extend between the epithelial cells and the surface; this tissue grows so as to form a very rich adenoid tissue. At the same time an active growth of adenoid tissue in the walls of the peribronchial lymphatics takes place; that is to say, there is a hyperplasia (Sanderson) of the pre-existing follicles, as well as a new formation. [The most active transformation of the pseudostomatous tissue of the bronchi into adenoid tissue I have met with was in rabbits suffering from chronic pyæmia; it has been already stated that the reticulum of branched cells which stretches between the epithelial cells of the surface is better developed in rabbits than in guineapigs, in the normal condition.]

(*c*) In the large bronchi, which have become involved in the secondary process, another noteworthy change takes place, viz. the fusion of groups of the germinating epithelial cells, not only those of the free surface, but also those of the mucous glands, so as to form multinuclear protoplasmic cylinders and lumps (giant cells).

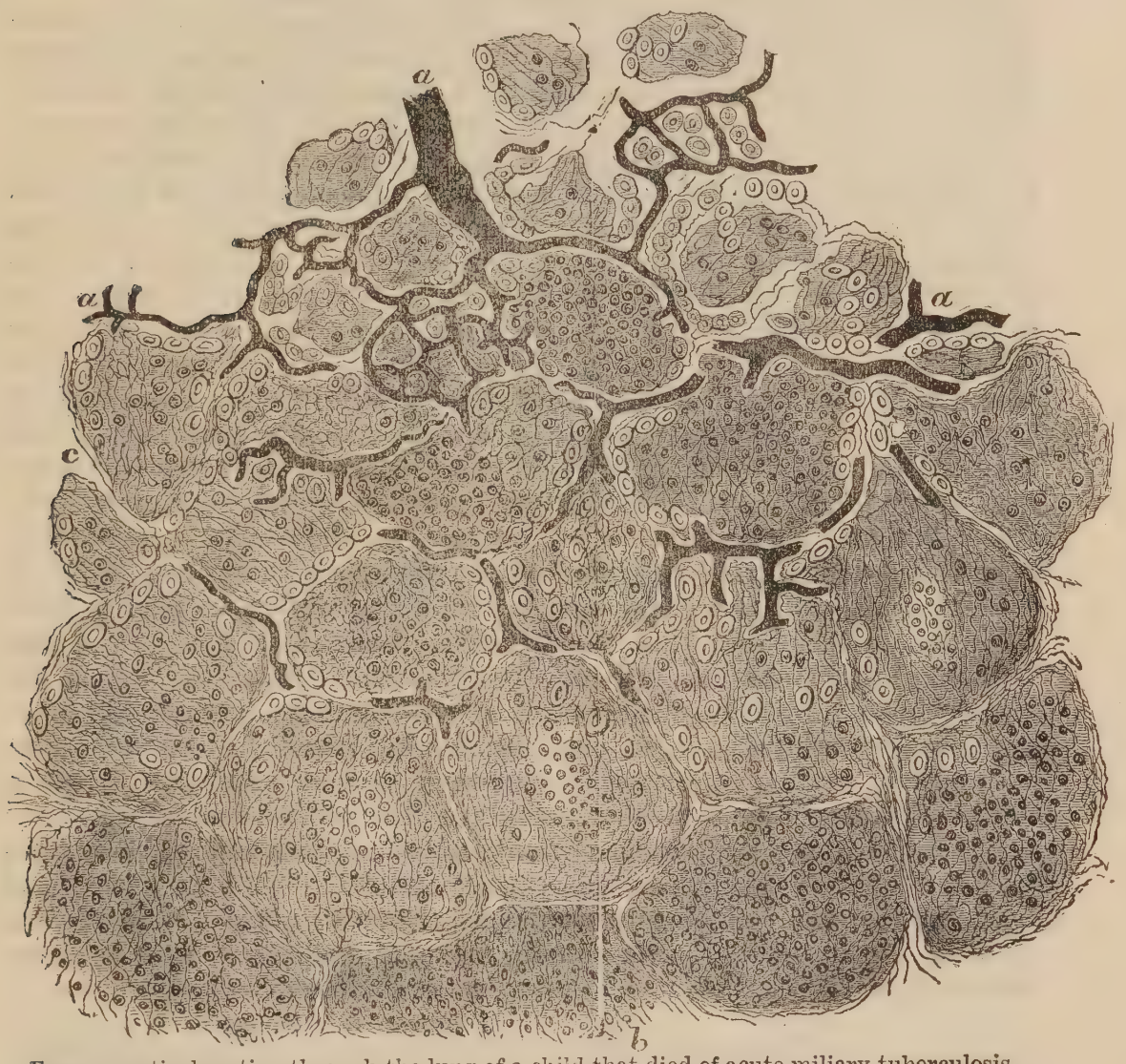
This secondary process affecting the alveoli and bronchi, which may be justly called the catarrhal pneumonic process, always accompanies artificial tuberculosis when it has extended to the interalveolar tissue; in the early stages of artificial tuberculosis, *only the perivascular lymphangeal cords are to be met with.*

If the infection has been established from the pleural cavity, the germination of the endothelium of the surface round the stomata, and the transformation of the subpleural lymphatics into cords of adenoid tissue, is the first symptom, and is followed by the appearance of perivascular adenoid cords. If, however, the lung becomes tuberculous by infection from the blood-vessels, the peritoneal cavity, or the subcutaneous tissue, the perivascular adenoid cords are the first structures that make their appearance. In lungs which have become the seat of chronic pyæmia, the first changes are to be found in the alveolar septa and alveoli, viz., the formation of patches and nodules similar to those that I have designated before as secondary; and if the process lasts long enough, those changes take place that I have designated before as primary tuberculous changes.

The opinion has been expressed (by Sanderson and Wilson Fox) that the process of artificial tuberculosis in the lungs of guineapigs resembles, in its anatomical features, the tuberculosis process in man. I will therefore examine the process that is clinically and anatomically known as acute miliary tuberculosis in man. For this purpose I shall describe the changes that I found in three series of cases of the disease in children, representing, as we shall see, three different stages of development. In the first series the lungs exhibited all the anatomical appearances of acute miliary tuberculosis. On microscopical examination it was found that the nodules were due to groups of alveoli (with the corresponding infundibula) being filled with and distended by a fibrinous material that contained granules and a few small cells; generally these

latter occupied the centre of the alveoli. The walls of the alveoli were hardly distinguishable; and the capillary vessels were not permeable, as shown by the fact that, in well-injected preparations, the injection did not penetrate into the capillaries of the alveolar septa. The alveoli next to these nodules contained the same fibrinous material; but they were not filled up by it completely; and their epithelium could be distinctly recognized, having become wholly or partially detached, the individual cells being somewhat enlarged, and some of them containing two nuclei. Here the injection material penetrated the capillary blood-vessels more or less perfectly; the alveoli of the neighbouring parts contained either a small amount of fibrinous material, besides isolated young cells, or a

Fig. X.



From a vertical section through the lung of a child that died of acute miliary tuberculosis. The figure represents about the third part of a tubercle.

a, Injected blood vessels, becoming quite impermeable towards the centre of the tubercle. *b*, Centre. *c*, Periphery of the tubercle.—Magn. × 160.

homogeneous gelatinous substance that had become stained with hæmatoxylin. The epithelium was very distinct, its cells granular. In some of the alveoli the epithelial membrane was more or less detached from the alveolar septa; the capillary blood-vessels were perfectly permeable.

In the second series of acute cases of miliary tuberculosis, in which the lungs did not differ in macroscopical appearance from those of the first series, but in which the process had lasted longer, the microscopical appearances were somewhat different. The nodules were seen to differ in their

structure from those in the former series in the following respects. In some of them it was easy to recognise that they represented a number of alveoli very much distended by a fibrinous substance similar to that described above, which included granular material and a number of small cellular elements ; the trabeculæ of these nodules (that is, the interalveolar tissue) were slightly thickened and contained young cells, their capillary blood-vessels being not completely permeable and not easily distinguishable. Besides these there were nodules of which only the central alveoli were in the state just mentioned ; whereas in those situated more peripherally the fibrinous material could no longer be discovered, but they were filled in one or other of the following ways :— First, by spherical nucleated elements, many of which could be still recognised as epithelial cells, by their size, granulation, and nucleus, and some of which contained two nuclei. In these places the interalveolar trabeculæ were thickened in a very marked manner, exhibiting all the appearances of an infiltrated tissue, that is to say, a more or less distinct reticulum of nucleated fibres, in the meshes of which lay small lymphoid corpuscles very readily stained by logwood or carmine. Or, secondly, they were filled by one large multinucleated mass or giant cell. In the latter case the giant cell, or rather the multinuclear protoplasmic cylinder, contained the nuclei either regularly distributed in its periphery, or all crowded together in the central part of the mass. As regards the nuclei, it may be said that they stain readily; they are relatively small, sharply outlined, and contain one or two nucleoli. The protoplasm of the giant cell is tinted slightly yellowish, does not stain in hæmatoxylin, and is very regularly filled with small granules of equal size. As regards the development of these giant cells and their nuclei, I must first contradict those authors who say that they originate generally by a free-cell formation in the veins, as well as those who make them originate in lymphatic vessels; for I have followed their development from the epithelial cells of the alveoli with all possible certainty. I have been able to make out that the whole epithelial lining of an alveolus becomes fused together into one protoplasmic lump, which fills the alveolar cavity, and the nuclei of which rapidly divide, remaining, however, in their original places, viz. peripheral. What we have before us is a protoplasmic cylinder, the transverse section of which shows a peripheral ring of nuclei. But a single epithelial cell may also become transformed into a multinuclear giant cell : an epithelial cell increases rapidly in size, probably at the expense of its fellows ; its protoplasm becomes enlarged as well as its nucleus, then this nucleus gives rise by cleavage, or by budding, to a number of small nuclei, so that it is transformed into a number of nuclei lying in the middle of the cell.*

Where the alveoli contain giant cells the alveolar septa are very much thickened, and are seen to consist of a tissue that contains branched and spindle-shaped cells, the substance of which has more or less the appearance of a fibrous tissue, their processes as well as their body

* I have little doubt that Klebs would be inclined to regard the very regular granulation of the giant cells previously mentioned, as being due to the presence of micrococci ; such an assumption, however, could not easily be proved. It does not follow because a substance is filled very regularly with granules that these are micrococci. To such view it may be objected, first, that there a number of normal tissues that appear after hardening to be just as regularly filled with granules (*e.g.* the liver-cells of any liver hardened in spirit), and, secondly, that the resistance of these granules to acids and alkalies after hardening does not prove them to be micrococci.

being slightly fibrillar. Between these there are very few lymphoid corpuscles to be found.

Fig. XI.

From a vertical section through the lung of a child that died of acute miliary tuberculosis. The tubercle represented in this figure is of a later stage than the one in Fig. X.
The tissue surrounding the tubercle is like adenoid tissue; the original alveoli contain giant cells, which give origin to nucleated fibres.--Magn. × 50.

In a third series of lungs, which also in macroscopical aspect did not differ from the former ones, it was seen that almost all the nodules contain giant cells, corresponding to the alveolar spaces. These, however, have undergone changes which are correctly described by Schüppel and others; *i.e.* the giant cells give rise to a network of branched nucleated cells, as well as to a few spherical nucleated elements that lie in its meshes. This network grows at the expense of the giant cell, which undergoes germination at the same time. We have here what is generally called a reticular tubercle (*see* Fig. XI.). From one giant cell a number of giant cells may take their origin.

The nearer to the centre of a nodule the giant cell lies, the more extensively and quickly does a transformation of its substance take place. It becomes converted into a very dense feltwork of fibrillar tissue, the nuclei of which gradually disappear, while the tissue itself dies away, becoming firm and hard, and finally resembling a granular substance, in which fibrils can be made out very indistinctly. While the network of the nucleated cells continues to grow at the expense of the giant cells, the process of necrosis spreads gradually to the peripheral parts. In this stage of the process the thickened interalveolar trabeculæ become also comprised in, and identified with, the tissue that originated from the giant cells. In the neighbourhood of the nodules there are very numerous places where the interalveolar trabeculæ are thickened and contain numerous young cells, the epithelium of the corresponding

alveoli being, at the same time, in a state of germination. In general the tubercular nodules of both these latter series have the common character that the peripheral zone of the tubercular nodule is a regular adenoid tissue, being composed of a delicate reticulum which includes small lymphoid corpuscles; this adenoid tissue is in continuity with the tissue of the interalveolar trabeculæ above mentioned. In these stages of the tuberculous process, we find numerous branches of large blood-vessels in the immediate neighbourhood of the nodules, provided with the same perivascular cords of adenoid tissue as have been described in the tuberculous lung of the guineapig.

Finally, it may be mentioned that these nodules also grow in circumference, by the alveolar septa of the neighbouring alveoli gradually becoming thickened, while, at the same time, the epithelium of the corresponding alveoli undergoes the changes before described. The capillary vessels of these parts show the same changes as were mentioned in the case of the lung of the guineapig, *i.e.* they are transformed gradually into nucleated fibres, which may be supposed to be, for a certain time, still permeable by coloured fluids.

If we summarize the results thus described, it is evident that the changes in the acute process of miliary tuberculosis in man are only to a limited extent similar to those which occur in the process of artificial tuberculosis in guineapigs. In the lung of tuberculized guineapigs the first structural changes may be briefly characterised as consisting in the appearance of perivascular lymphangeal nodules, whereas the changes of the interalveolar tissue and the alveolar epithelium form only a secondary process. In miliary tuberculosis of man, on the other hand, we see that the first changes take place in the alveoli and interalveolar septa, and these changes are followed by the appearance of perivascular cords.

It is therefore probable that, in artificial tuberculosis of the lung of the guineapig, the parts first attacked are the small branches of the pulmonary artery or pulmonary vein, whereas in acute miliary tuberculosis of man the capillary blood-vessels of the alveoli seem to be the tissue from which the action of the morbid agent starts.

No. 4.

Anatomical Research towards the Ætiology of Cancer, by Dr. Creighton.

Various meanings of the term "cancer."

The name "cancer" as applied to a kind of tumour contains in it no definition of the object. According to the current explanations of its origin the name is purely fanciful. It is said by some writers to have been applied to certain tumours of common occurrence to signify their gradual and persistent progress. According to others, the name originated in the resemblance of the spreading veins, often seen in the cancerous breast, to the extended claws of the crab. Virchow, who quotes the latter explanation, supports it by the fact that the condition of *pannus*, depending on the unwonted appearance of branching blood vessels in the cornea, was at one time also known as carcinoma. In the latter view the name connotes by a round-about way, a property of the disease that is entirely accidental; in the former view, the name conveys also in a metaphorical form, the sum of qualities implied in the term "malignant;" and it is no doubt on account of this figurative meaning precariously associated with it, that the name of cancer is still retained in the scientific classification of diseases. From its metaphorical nature it is, however, quite unfitted ever to become a general name in medicine connoting a certain definite assemblage of attributes. As a figurative expression it implies with sufficient fullness all that is implied in the term "malignancy," and its proper function may be considered to be that of an additional and colloquial name for all tumours whatsoever that prove to be malignant.

But unfortunately as tumours have come to be more thoroughly investigated, the term cancer has had assigned to it by courtesy a certain definite connotation quite foreign to, and not at all suggested by its etymology. Pathologists chiefly in Germany have retained the figurative name of cancer, side by side with such anatomical terms as enchondroma, myxoma, &c., as the generic name of a greater part of the new growths that have an epithelial type of structure. This arrangement, illogical in itself and tending to the confusion of students, came about naturally enough. At an early date in the study of disease, the word cancer in one form or another was applied to several very distinct diseases, including *pannus* (carcinoma), already mentioned, noma (*cancer aquaticus*), and chancre. These came to be detached from the cancerous group before the time of accurate microscopic investigation, but since the discovery of the different tissues of the body, the process of detachment has been very much extended. The law of Johannes Müller that all tumours correspond in their structure to one or other of the various tissues of the body, formed the basis of a scientific classification of tumours. Müller himself erected the class of enchondromata. Hannover applied the term epithelioma to a class of tumours also known as cancroid, while Virchow defined the new classes of myxomata, gliomata, and most important of all, sarcomata. The latter term had previously existed as it were in rivalry with the term cancer, and was applied with equal uncertainty. Thus J. F. Meckel spoke of a "pankreasartiges Sarcom." Unlike cancer, however, it did connote certain objective characters of new growths, and on this ground it has been retained by Virchow as being capable of a scientific definition. It implied originally a kind of structure resembling exuberant granulations, and inasmuch as

the new growths developing from cells of the connective-tissue series are supposed to pass through a stage of "indifferent granulation," this common point in their history has been laid hold of as the limited generalisation which shall be conveyed in the name.

Thus there have been drafted off under new classes a very large number of the heterogeneous assemblage of tumours at one time known as cancerous. The residue are tumours of an epithelial type of structure, including some that had from the first been considered as pre-eminently cancerous, such as tumours of the female breast, and of the stomach; and for this residue the original unscientific and fanciful name has been retained. The question remains whether the term cancer should not rather be employed as a general synonym for malignant tumours, when such are spoken of solely in respect of their malignancy and without reference to their histology; that is to say, as a convenient additional name implying no more than is implied in the original metaphor. In this country modern usage has practically given this signification to cancer. But if cancer is to be employed in this sense, and not as in foreign writings, also in the histological sense, it remains to find a scientific term which shall be a name for new growths of epithelial origin, as sarcoma is a general name for tumours of a connective-tissue origin.

There is, however, a fundamental want of agreement as to whether new growths of an epithelial type of structure do actually develop from epithelium. Professor Virchow holds that the all-pervading connective tissue is the *matrix* of these as well as of the connective-tissue growths proper, just as the school of pathologists, which it is his merit to have superseded, held that an all-pervading blastema was the *matrix* of every new growth. Till there is more agreement on this point, a scientific name for the tumours of epithelial-like structure, based as it will necessarily be on their histogenesis, must be postponed.

Unsuccessful attempts to produce cancer artificially.

In making a systematic investigation of points in the histogenesis and causation of malignant tumours, it was obvious at the outset that the investigation would be facilitated very much if such tumours could be produced artificially. Not only would the channels of infection, and perhaps the agents of infection, be discovered, but the disease itself, the product of the infection, might be obtained at the various stages of its development, and, to speak generally, under conditions that could be varied to suit the requirements of the research. Attempts were therefore made to propagate cancerous tumours artificially, by means of the injection of the cancerous juices, and by the grafting of portions of tumours among the tissues of an animal. Previous experimenters having failed in the attempt to graft portions of cancerous tumours from the human subject on dogs, occasion was taken to graft from one dog to another, and, in the case of a mammary tumour in the cat, from that animal to another of the same species. The experiments in dogs failed in several cases from the difficulty in retaining the graft in the animal designed for infection. In two cases, however, thin slices of the granulating surface of mammary tumours were applied to previously prepared granulating surfaces of the subcutaneous tissue in other dogs, and successfully retained by stitching the skin over them. In both cases no growth at the point of grafting took place, but a thickening under the cicatrix, corresponding to the size of the graft introduced, could be felt for some weeks after. A dissection was not made of these cases, but there is no doubt that the grafts, as in former experiments where portion of tumours of the human subject were grafted on animals, became surrounded by a fibrous investment as if they had been foreign bodies and incapable of being incorporated with the surrounding tissues. The

injection of the cancer juice under the skin was tried in several cases without effect. Both in these and in the grafting experiments care was taken that no interval should elapse between the removal of the tumour and the commencement of the experiment, so that the tumour elements used for inoculation presumably retained their vitality. These experiments were very limited in number, for the case of the inoculation of tubercle appeared not to be an analogous case, and beyond the supposed analogy of tubercle there was nothing to indicate the conditions under which cancer might be produced.

Microscopic investigation of secondary malignant tumours in the liver.

Recourse was then had to the microscopic examination of new growths with material procured in the ordinary way of *post-mortem* examination. Reversing the order of sequence, secondary tumours, chiefly in the liver, and also in the lung, kidney, and serous membranes were first made the subject of an investigation.* It seemed probable that the minute examination of secondary tumours in the liver, in a series of cases where the primary tumours were of various texture, would throw some light on the common element of malignancy to which they owed their origin. The liver was, as it were, neutral ground, in which the malignancy of the parent tumours, whether they were cancerous (epithelial), sarcomatous, or lymphomatous, manifested itself. The tumour-nodules in the liver were further recommended for investigation by a circumstance of the first importance in an investigation having reference to histo-genesis. Although it generally happens, in cases where secondary tumours are found, that the disease as a whole has fully developed itself, and has been indeed the cause of death, it is not uncommon to find the secondary nodules in one organ or another in all stages of development, from the most initial up to the most advanced. Thus, in one of the cases to be hereafter referred to, the lungs contained secondary tumours so broken down as to be hardly available for minute examination, whereas the liver contained only two or three very small nodules, which were presumably, from their size and isolation, the very earliest manifestation of the disease in that organ.

Enumeration of cases.

The tumour-nodules in the liver occurred as secondary growths in the following cases:—

1. Cancerous tumour (of tubular gland structure) of the ascending colon, in a horse examined *post-mortem* at the Brown Institution. The nodules in the liver were numerous, and varied in size from a billiard ball to a small nut. The peritoneum, chiefly of the diaphragm, and also of the abdominal parietes, was the seat of numerous small nodules, often fused into cords.
2. Spindle-celled sarcoma (melanotic) of the front of the leg, back of the shoulder, and other parts of the subcutaneous tissue, in a female patient under the care of Mr. Croft in St. Thomas's hospital. The liver nodules were numerous, and of various sizes. Some of them were marked with melanosis, and others presented the usual whitish appearance of liver tumours. Large melanotic tumours, very much softened, were found also in the axilla and at the root of the lung. The pleura contained a good many small melanotic nodules.
3. Myxo-sarcoma growing from the posterior aspect of the lower end of the femur in a male patient, aged 24, under the care of Mr.

* The present account is limited to the observations in the liver. I may be stated, however, that strong confirmatory evidence was derived from several cases of secondary growths in the lung.

Le Gros Clark, in St. Thomas's Hospital. The liver nodules were three in number, from $\frac{1}{2}$ to $\frac{1}{4}$ inch diameter, situated in the upper periphery of the organ. The lungs and pleuræ contained several osseous masses, some of them as large as a walnut.

4. Lymphomatous tumours of large size in the spleen of a dog examined *post-mortem* at the Brown Institution. Attention was drawn to the liver by several small white nodules near its surface the size of a pin-head. The thyroid body on one side was greatly enlarged and otherwise changed.

5. Large spherical white tumours in the liver similar to those found in the spleen in No. 4, also in a dog examined at the Brown Institution. No seat of disease, that could be called primary with reference to the liver tumours, was found.

6. Tumour of the upper jaw, of soft consistence, and containing many giant cells, in a male patient, aged 30, under the care of Mr. Simon, in St. Thomas's Hospital. The secondary disease was very extensively distributed within the abdomen and in the lungs. The liver tumours were numerous, and varied in size from 2 inches diameter to $\frac{1}{4}$ inch.

7. Cancer of the rectum in a patient who died in Charing Cross Hospital. The liver nodules were many of them in an early stage of development. (The material in this case was preserved by Dr. Bruce.)

8. Cancer of the neck of the womb in a patient under the care of Dr. Barnes, in St. Thomas's Hospital. The liver nodules were few in number, and very small.

9. Cystic disease of the ovary in a patient under the care of Mr. Sydney Jones, in St. Thomas's Hospital. The liver contained one spherical nodule, about $\frac{1}{2}$ inch in diameter, abruptly marked off from the surrounding tissue, but wanting the white appearance of an actual tumour nodule.

10. Whitish masses in the liver of a dog, of uncertain origin. (This case is not introduced as a case of malignant tumour, but for purposes of illustration.)

Analysis of the cases.

In cases No. 4, 9, and 10 the nodules in the liver are to be considered as in the initial stage, and, when taken by themselves, as of doubtful origin and destiny.

In cases No. 1, 2, 6, and 7 the secondary tumours had a remarkable resemblance to the primary, not only in the form and size of the cells, but also the arrangement of the stroma, where such existed (1, 6, and 7).

In case No. 3 the primary tumour had at certain points a well-marked myxomatous structure, while at other points the arrangement of cells and fibres was more nondescript. The nodules in the liver, which were rudimentary, resembled more the nondescript portions of the primary tumour than the myxomatous portions of it.

In case No. 8 the primary new growth at the neck of the uterus was not obtained for examination.

In case No. 5 the liver tumours could not be referred to nor compared with any primary growth (source of infection).

It will thus appear that in five cases there is complete evidence of the identity of the secondary tumours with the primary. The nodules in the liver were composed of large spindle cells, of giant cells, of tubular

gland structure, &c., resembling the parent tumours respectively, and this resemblance extended even to very minute particulars.

Current opinions on the mode of origin of secondary tumours.

The current explanations of the origin of secondary tumours in the liver may be classed under two heads :—1st, those which ascribe the new growth to the multiplication of tumour cells that have wandered to the liver from the primary tumour, and have become centres of cell-proliferation ; 2nd, those which ascribe the new growth to a transformation of certain pre-existing elements of the part. The evidence that will be adduced below is entirely in favour of the latter explanation, and will suffice to show what particular elements are transformed, and what is the particular manner of their transformation. The most important statements as regards general pathological doctrines that have been made hitherto on this point are those of Professor Virchow, and it will be necessary to quote them briefly.

The reference to new growths in the liver is preceded by the following statement of one of the cardinal doctrines of the Cellular Pathology : —"Bearing in mind that the lymphatic tracts, in so far as they are "concerned, have a close relation to the connective tissue, we should "still not be in error if, *instead of plastic lymph—the blastema of* "*earlier writers, the exudation of later—we were, with a few reserva-* "*tions, to take the connective tissue, with its equivalents and adjuncts,* "*as the chief matrix-tissue of the body*, and to trace therefrom the "development of the greater part of new formations."

The application of this doctrine to the liver, is summed up in the passage that immediately follows the sentence just quoted :—"If we "take the case of a particular internal organ such as the brain or the "liver, it were difficult to conceive of the formation of a new growth "without the intervention of some special formative material, so long "as one recognised nothing in the brain besides nerve substance, and "in the liver nothing but liver cells and vessels ; for one may be easily "convinced that, as a rule, the new growths in the liver do not proceed "from the liver cells or the vessels. . . . But I had first to make "out that there were connective-tissue cells in the liver and interstitial "neuroglia cells in the brain, which are the equivalents of the ordinary "connective-tissue corpuscles. In fact it appears to us, as Reichert "was the first to suggest, that the groundwork of the body is made up "of a more or less continuous mass of connective-tissue elements, in "which, at certain points, other things, such as epithelium, muscles, "vessels, and nerves, are set. It is in this more or less connected "framework that the majority of new growths, according to my in-"vestigations, take origin, and that, too, according to laws the same as "regulate the embryonic development."*

The summary of facts to be given below is put forward as strong evidence that the liver cells, the parenchymatous or predominant cells, and not the cells of the connective tissue of the organ are the elements that undergo transformation. And this evidence will serve among other purposes as a test of the particular doctrine of the cellular pathology that is here quoted.

The process is a transformation of liver cells by vacuolation.

The transformation that the liver cells undergo consists in a vacuolation of their protoplasm, with other associated changes. A description of the usual forms of vacuolated cells met with in the cases above enumerated will first be given. Secondly, it will be attempted to show that

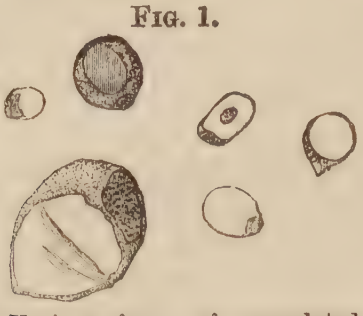
* Cellular Pathologie, p. 485.

the vacuolation of liver cells stands in a causal relation to the formation of tumour nodules. Thirdly, the nature of the vacuolation process and its significance as regards histogenesis will be discussed. Fourthly, the affinities of the vacuolation process, as observed in the formation of secondary tumours, to certain forms of cell change in the normal life of the organism will be pointed out.

Various degrees of vacuolation.

A typical example of a vacuolated liver cell has the appearance of a signet ring. The substance of the cell is excavated to form a vacuole or vesicle, at one side, or on one pole of which is accumulated a mass of protoplasm either of crescentic shape with its two extremities merging gradually in the walls of the vacuole, or as a circumscribed oblong or round lump. It is a question, chiefly of terms, whether the peripheral mass is the nucleus or the substance of the nucleus in an excentric position, but it is an invariable property of this mass, whatever its form, that it is capable of deep staining (by logwood, for example) and that it contrasts in this respect with the rest of the cell. The signet ring form of vacuolated cell here described has been called typical, but a modification of it, in which there is the addition of a round cell, also capable of deep staining, lying free in the vacuole, may be described as a more perfect type. (In describing these forms as typical, it is intended only to make them available as standards of comparison.) In some cases the deeply-coloured peripheral mass is very much larger than in others, and the vacuole proportionately smaller. In other cases, again, the coloured protoplasm is not massed at one point of the circumference, but is, as it were, distributed equally round the whole of it, forming a substantial cell wall. This is chiefly seen in cases 4 and 10, and these cases are so peculiar in this respect that they will be reserved for separate consideration. In still another form or degree of vacuolation, nothing is seen of the original cell but a faint ring, quite uncoloured by the staining fluid, and containing within it a round cell which, on the other hand, is deeply coloured.

Fig. 1.



Various forms of vacuolated cells occurring among the tumour cells. The large cell at the left lower corner is from case 6 (giant-celled). (Hartnack, Oc. 3, Obj. 7.)

The different kinds of vacuolation here described are evidently modifications of one and the same process.

Contents of the vacuoles.

As regards the contents of the vacuoles the observations are defective. The specimens used for the investigation were preserved for a considerable time in spirit, and the sections of them were mounted in a solution of Canada balsam. After this preparation the vacuoles had generally the appearance of being empty, as if their contents had been dissolved out, and it is highly probable that they were originally occupied by fat. In a number of cells in cases 3, 6, and 7, the vacuoles were filled with a fluid substance which became stained by the logwood in the same way as mucin (for instance in the thyroid body), and in other cases they presented the appearance of being filled with a thin granular protoplasm. The question of contents will be considered again later. It is sufficient for the present to direct attention to the deeply-coloured masses of protoplasm associated with the vacuolated cell in one form or another, for these are factors that can be reckoned with by themselves and independently of the accompanying fluid products.

Vacuolation casually related to production of tumour-nodules.

The above description of vacuolation is taken from liver cells still distinguishable as such, grouped either in acini or cylinders, or lying

more or less isolated. It has next to be shown what reason there is for supposing a connexion to exist between the process of vacuolation and the formation of the tumour cells. An *à fortiori* proof of this will be given in describing in detail the manner in which particular vacuolated cells give rise to tumour cells, but there is a certain amount of general evidence of the relation between vacuolation and the growth of secondary tumours, which may be stated in the first place.

Primâ facie evidence.

The most instructive case for this purpose is the fourth in the above list. The greatly enlarged spleen contained several large white tumours of a well-marked lymphomatous structure. They were spherical in shape, some of them projected about an inch above the surface, and they were all definitely circumscribed from the surrounding splenic tissue. The liver contained a number of small white pin-head nodules, several of them visible on the surface. On section through one of these it was found that the liver tissue, for a radius of about $\frac{1}{4}$ inch round the small white nodule, and to a depth of $\frac{3}{4}$ inch, had a colour different from the rest of the liver. This area of altered liver tissue corresponded exactly to the usual cup-shaped outline of a tumour nodule in the periphery of the liver, and on microscopic examination it was found that it was an area of general vacuolation of the liver cells, and that it was definitely bounded from the adjacent liver tissue, which was normal. It is impossible to regard this appearance otherwise than as the initial stage of a new growth, such as was fully developed in the spleen. Again, in case 1, there appeared round a white nodule a zone of tissue of less marked whiteness. This zone was found to be liver tissue in a state of vacuolation, having already acquired a certain resemblance to the more developed tumour tissue that lay within it. In case 2 (spindle-celled sarcoma) nodules were found divided into smaller areas by bands of fibrillar tissue. Among the areas composed of spindle cells there occurred one or two also marked off by the fibrillar septa, which were composed of cells still distinguishable as vacuolated liver cells.

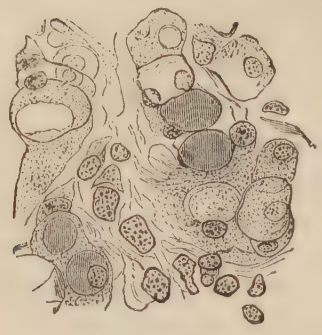
Such instances as these serve to establish a *primâ facie* connexion between the vacuolation process and the growth of the tumour. The exact nature of this relationship will now be described. It may be stated at the outset that it is not to be seen with equal clearness in all the cases enumerated. Case 8, for example, would not probably of itself have suggested such an origin of the tumour cells, but taken along with the others it brings a certain amount of confirmation. The most instructive sections were obtained from cases 1, 2, 3, 6, and 7.

Particular evidence.

It may be said, in general, that the deeply stained mass of protoplasm remaining from the vacuolation of the cell, whether it occupies the periphery of the vacuole or exists as a free cell within it, is the first stage of the tumour cell, which henceforth enjoys an independent existence, and develops, so to speak, on a new departure. The varieties of its origin are naturally as numerous as the modifications of the vacuolation process. Where the latter is so extensive that only a small round cell survives within the faint outline or skeleton of the original liver cell, the tumour element commences at the lowest stage of "indifference," or the granulation stage, and may either go on to attain the characters of a cell in the primary growth, or, as there is reason to suppose, it may fall short of this. Coming next to the signet-ring type of vacuolated cell, two modifications in its progress towards a tumour cell are found. In the first of these the peripheral mass becomes detached from the more or less slender ring of the vacuole, which in its turn atrophies or disappears in one way or another. It may be well to notify

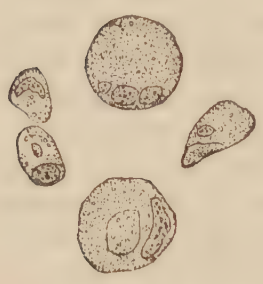
here that in describing the steps of this process, as in the description of all processes of cell-change from *post-mortem* appearances, one is obliged to speak as if the successive steps were observed in the same element. What is observed is, of course, a number of cells of the same kind in different stages of the same process. The subsequent progress of these disengaged masses, as well as of the small round cells described as the first variety, may be made out with tolerable certainty. It is of course evident from their property of deep staining that they are destined to a development of some kind. The particular evidence that they become tumour cells consists in the fact that they are found in all transition stages, and in such proximity as to make the relationship not at all doubtful. But it is in the other varieties of the vacuolation process that the evidence of identity is most direct. In a very large number of the cells, especially in cases 3 and 6, the outline of the vacuole persists. These are cases of the signet-ring type of vacuolation in which the wall of the vacuole is a more or less substantial prolongation of the crescentic peripheral mass, and capable of a faint degree of staining. The vacuole, as has been said, does not break up, as in the former case, but remains attached to the chief element in the new growth, the peripheral mass, and can be identified in the mature tumour cell. What was the vacuole becomes filled with a pale granular protoplasm, the highly coloured peripheral mass remaining in the same relation to it as it did to the vacuole while the entire cell could still be called a liver cell. An appearance observed several times (in cases 3, 6, and 7), is probably to be considered as the stage intermediate between the first vacuolation of the liver cell, with its probably oily contents, and the stage in which the vacuole was filled with a pale protoplasm. In these cases, which have already been mentioned, the vacuoles were filled with a fluid which coloured in the same way as mucin, and was therefore to be considered albuminous.

Fig. 2.



From the liver in case 3. Vacuolated liver cylinders, occurring side by side with the mature tumour cells, and showing production of small round cells. (Oc. 3, Obj. 7.)

Fig. 3.



Cells from various liver tumours, giving the appearance of vacuoles that have been filled with granular protoplasm. (Oc. 3, Obj. 7.)

There was no doubt of the identity of these cells with liver cells, on the one hand, for they occurred in unmistakeable liver cylinders, and there was equally little doubt as to their relationship to tumour cells on the other hand, for they occurred (in very young nodules) in the midst of tumour cells in all stages of development. The occurrence of an albuminous fluid in the vacuoles suggests that if, as is probably the case, the original contents in the case of liver cells, are of an oily character, a substitution takes place of an albuminous fluid, which in its turn assumes a more or less solid protoplasmic character. A similar substitution has been observed in the subcutaneous fat and in the medulla of bone. In many cases the protoplasm which the peripheral mass attracts to it or secretes, is disposed not only within the outline of the old vacuole, but also as a fringe round the entire cell. But in a very large number of cells, in cases 3 and 6 (both sarcomatous), the appearance of vacuolation, depending as it does on the deeper colouring of the outline of the cell and of the peripheral mass, remained after the

contents of the vacuole had ceased to be fluid, and afforded, at the first glance, a remarkable proof of identity.

The giant cells that occurred in great numbers in case 6, and to a lesser extent in case 3, are interesting in themselves, and will be spoken of again when the nature of the vacuolation process is discussed. For the present purpose they serve to show clearly the relation between the liver cells and the tumour cells. The mature giant cells of the tumour contained nuclei generally varying in number, in proportion to the size of the cell, from two or three up to 20 or more. The nuclei were not less in size than a white blood corpuscle, and a cell with many nuclei formed, therefore, a very large object. In several of the cells the nuclei were arranged round the circumference of the cell like a necklace, leaving the centre free. Cells of this size were seen among the liver cells, sometimes grouped together and facetted like a cluster of liver cells, having the characteristic brownish protoplasm of the liver, and differing in no other respect from the cells of the organ, except in their size and the occasional absence of a nucleus. The next stage was represented by cells evidently corresponding in size and general appearance to the former, in which the entire substance of the cell was occupied by a number of clear round or oval vacuoles. In one cell a coloured nucleus, presumably the original nucleus of the liver cell, was observed at one end, having its border scooped out at various points corresponding to the segments of vacuoles clustered round it and encroaching upon it. The nucleus had thus a stellate appearance, with its rays or processes separating for some little distance the vacuoles grouped round it. The last stage is the mature stage of the giant cell, where the vacuoles are no longer visible, but instead of them coloured nuclei, apparently to the same number and in the same positions. In others of the giant cells, belonging probably to the intermediate stage, vacuoles were seen containing small round cells in their centre, with a greyish granular protoplasm filling up the rest of the space. In still another form each vacuole had a crescentic coloured mass of protoplasm on one side of it.

Fig. 4.

Cells from case 6 (giant-celled tumour) showing various stages of development, *b* representing a stage intermediate between *a* and *d*. (Oc. 3, Obj. 7.)

In the cases from which the preceding descriptions are drawn, the various modifications of the vacuolar process were generally present together. Thus, among the giant cells there occurred cells of the ordinary signet-ring type, while in case 3 there occurred along with cells that retained the outline of the vacuole, others in which the peripheral mass alone developed. The variety in which only a small round cell survived the vacuolation occurred in nearly all the cases. It frequently occurred that in the midst of an area of more or less developed tumour cells with bright colouring there were found greyish patches with a number of small coloured cells strewn over them. On closer examination the ground substance or soil of these patches was found to consist of the pale, ring-like skeletons of vacuolated liver cells, within many of which lay the small, coloured, round cells corresponding to those spoken of before as "indifferent," or granulation cells. It is possible to infer from these appearances either that all the rest of the tumour had passed through the same stage, and that those particular parts were backward

All the degrees of vacuolation may be found in the same case.

in their development, or that in some parts of the liver tissue undergoing transformation the cells had undergone a greater degree of vacuolation than in the others, and had been first reduced to the "indifferent" or granulation stage before taking on the characters of tumour cells. From the independent proofs given above the latter explanation is the more probable. An impression also conveyed by these same patches of vacuolated cells is that the number of round cells resulting from the process is smaller than the number of liver cells involved, and it is at least probable that the products of vacuolation not unfrequently abort.

Other issues of the vacuolation process.

Again, though it is quite certain that those indifferent round cells do very generally develop into mature tumour cells, there is good reason to suppose that in some cases they terminate in a fibrillar or adenoid kind of tissue, which may be called cicatricial, in contrast to the tissue of the new growth. The evidence in favour of this notion is derived from case 1, and more especially from case 2, a spindle-celled sarcoma. In the latter certain bands of fibrillar tissue are seen to merge with areas of small round cells, the relation of which to the liver cells on the one hand and to the tumour cells on the other rests on independent evidence. Another observation in connexion with these fibrillar bands of tissue is that they sometimes contained, as if imprisoned in their midst, small clusters of liver cells in a state of vacuolation. We are thus brought face to face with the notion, that not only the vacuolation of liver cells can issue in the formation of a fibrillar or cicatricial tissue, but that the same vacuolation process which in one group of liver cells may give rise to tumour elements may, in an adjoining group of liver cells, give rise to the comparatively safe product spoken of as fibrillar or cicatricial.* It is to be observed, however, that this, in so far as it is proved to occur at all, can only occur where the vacuolation has been of the extreme kind, and where the surviving product is a small round granulation cell.

The structure of the lymphomatous tumours shows an approximation to the above-mentioned fibrillar tissue, while they still retain their character of new growths in the strict sense of the term. In case 4, described already has showing a *primâ facie* case for the connexion of vacuolation and the growth of secondary tumour, the vacuolation of a tract of liver cells was of such a kind that the resulting appearance was that of a coarse network, in the meshes of which lay round cells, more numerous at some parts than at others. A later stage of the development was not seen in this case, and in the other case of lymphomatous tumours (No. 5) the same early stage was not observed. The evidence, therefore, taken as it stands, is not conclusive, but there is nothing hazardous in supposing that the coarse network, with the small cells in its meshes, as seen in No. 4, would have subsequently assumed the character of true lymphomatous or adenoid tissue. In this case the fibrous network was formed by the substantial coloured walls of the contiguous liver cells. That the network was formed actually by

* The observations of Holm and of Hüttenbrenner on the process of repair after wounds of the liver are of interest in this connexion. The cicatricial tissue was found to be derived from the liver cells, and the first stage of the reparative process was found to be a filling of the liver cells with fat. It has also been contended that the connective tissue in cirrhoris of the liver is derived from the liver cell, and the associated presence of fat in the liver cells has been remarked. Among others, Dr. Wickham Legg, (St. Bartholomew's Hospital Reports, 1872,) argues for the development of the connective tissue in cirrhosis from the liver cells, but the so-called "fatty infiltration" does not enter into his own description of the process.

vacuolated liver cells in close apposition, and not, for example, by the *tela conjunctiva* of the organ, was quite clearly shown by the fact that there occurred in the same preparations, and contiguous with the network, liver cells in which the vacuoles were smaller and the peripheral masses larger, and all gradations of the process.

The process of transformation in the liver cells has hitherto been spoken of from a purely morphological point of view as vacuolation, and the evidence has necessarily taken the direction of identifying particular stages of the vacuolation process with particular appearances in the tumour cells. It is now proposed to examine the nature of the vacuolation process, and its significance as regards histogenesis. This is the third of the points set down for discussion, and it may be disposed of briefly.

Vacuolation, as described in the preceding account, is the process that is known as endogenous cell formation. The signet-ring type of cell, or that form, with the addition of a free round cell in the vacuole, may be considered as a diagramatic representation of endogenous cell formation. Professor Virchow has described exactly the same forms of cells under the name of "physaliphoren," in order to distinguish their vacuoles from such transient vacuolar appearances as occur, for example, in a pus corpuscle, or a white blood corpuscle, during its amœboid movements. These are described as a variety of the endogenous mode of cell formation. It is scarcely necessary to illustrate this point at greater length.

Vacuolation, as here described, is the process of endogenous cell formation.

The only other probable view is that vacuolation is a form of necrobiosis of the cells in which it occurs, and as regards the vacuolated liver cells, which presumably contained at one time fat in their vacuoles, such an opinion would no doubt occur to those who think, without discrimination, that the mere presence of a fat globule in a liver cell is an evidence of its decay. It may be an evidence of its decay as a liver cell, but it is no evidence that it has lost any of that plastic force or power of transformation which is inherent in it as an element of the body.

It seems certain, however, from appearances observed in the cases forming the subject of this research, that a certain proportion of vacuolated liver cells do vanish entirely, leaving no product, which fact has been adverted to above, when it was said that the products of vacuolation in some cases aborted. But in the great majority of vacuolated cells, the one conspicuous and important thing is the deeply-stained and therefore vigorous mass of protoplasm that survives the vacuolation in one form or another. Even in those vacuolated cells in which the form and substance of the original cell have all but disappeared, the small round cells appear as bright coloured masses within the still obvious outline of the vacuole. This frequent and characteristic appearance suggests nothing so much as that the highly coloured and active young cell had sprung, as it were, from the ashes of the old one.* So long as such products result from the vacuolation, that process cannot be called one of necrobiosis.

Before leaving the subject of vacuolation regarded as the process of endogenous cell formation, the giant cells that occurred in cases 3,

* The appearances figured and described by AxelKey and Wallis (Virchow's Archiv., Vol. 55,) as occurring in the corneal corpuscules after inflammation, set up by pencilling with lunar caustic, are very similar to those here referred to, although a different interpretation is put upon them.

and 6 may be again referred to. Although the phenomenon of giant cells is sometimes attributed to a tardy or sluggish division of the protoplasm in the ordinary hyperplastic mode of cell growth, viz., by division, yet in the present case it was obvious both from the circumstances in which they were found, and from the presence of one or more vacuoles in them, that they were a product of endogenous cell formation.

The endogenous mode of cell growth is contrasted with the usual mode of cell-formation by division, and is frequently exemplified in the vegetable kingdom. If cell-formation by division is the hyperplastic process, wherein the new cell is merely a repetition of the old, the endogenous mode of cell-formation is the metaplastic process, in which the product departs from the type of the parent cell and acquires differential characters of its own.*

In the above cases, the results of the vacuolation process are found to correspond with the results to be looked for where the endogenous mode of cell-growth is resorted to. In one case the resulting cells became large spindle cells, in another case they became giant cells, in another columnar epithelium, the mature product in each case being different in type from the cell that produced it. The law of the continuity of the tissues, which would have been observed in the purely hyperplastic mode of cell formation, is, so to speak, abrogated by introducing the factor of endogenous cell growth; so that liver cells become the parents of sarcomatous cells or epithelial cells indifferently, and the epithelial cells, though belonging to the same group as the liver cells, are no more the hyperplastic products of the latter than are the spindle cells.

Summary of the argument.

To summarise the preceding statements, it may be said that secondary tumours in the liver originate in certain changes that go on in the liver cells simultaneously at various points, and that the efficient cause of their formation is the substitution of the endogenous or heteroplastic mode of cell-growth, whereby an alien product takes origin, for the normal plastic activity of the liver cells, whereby the organ is either maintained at a certain point or at the most undergoes a simple hyperplasia. As the ground in which tumours of very various characters may develop, the parenchyma of the liver may be regarded as a sort of indifferent matrix whose products owe their form, not to any influence that can be imparted to the liver cells as such, but to an extraneous influence. The mechanism, again, in nature by which this result is brought about, and by which this extraneous influence operates is the process of endogenous cell-formation.

The extraneous influence is, however, an assumption, and it now falls to be considered what reason there is for assuming the existence of such an influence.

The relation of primary tumours to secondary.

It has been already stated that the tumours in the liver had generally a remarkable resemblance, even in minute particulars,† to the tumours in other parts of the body, which have hitherto been spoken of as primary. These primary tumours differ from the secondary in the important respect that their elements resemble or at least are histogenetically the equivalents of the cells proper to the situation in which they grow. Thus, the sarcomatous tumours (2, 3, and 6,) originated in connective tissue structures, while the epithelial tumours (1, 7, and 8,)

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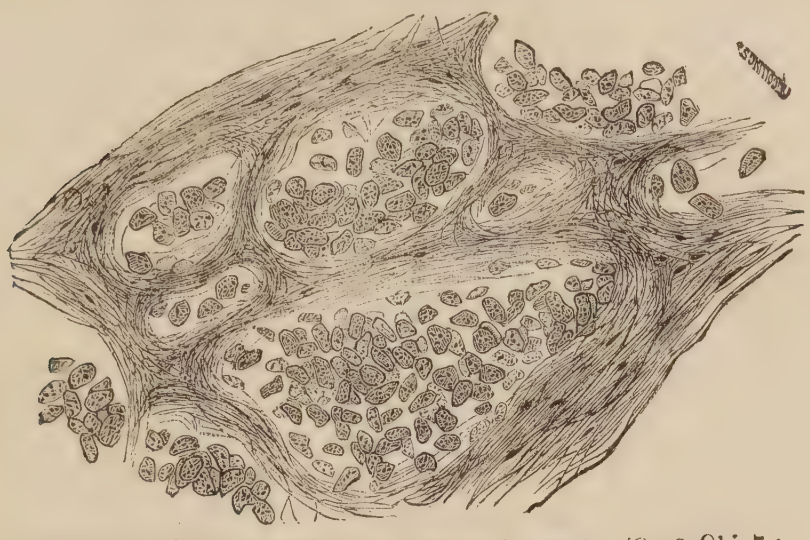
* Virchow, Cellular Pathologie, p. 491.

† The mode of development of the characteristic stroma in the various tumours has been omitted for the present.

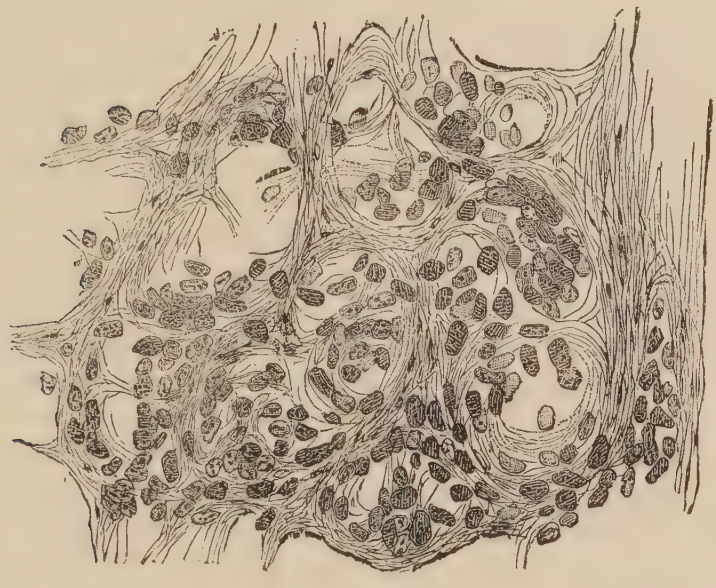
originated in mucous membrane. There is here, then, a fundamental difference between the primary and the secondary tumour, and there is no doubt also that the primary is the first in point of time. Admitting,

Fig. 5.



From the primary growth in the rectum, in case 7. (Oc. 3, Obj. 7.)

Fig. 6.



From a secondary nodule in the liver, in case 7 (cancer of the rectum). Same magnifying power.

then, that the tumours in the two situations are not two coexistent growths having their origin in a common cause, and that the one is "secondary to" or dependent on the other, the relation of parent and offspring must be admitted at the same time. The resemblance between the primary and secondary tumours is comparable to no other relation in nature than that of parent and offspring. The extraneous influence, therefore, which is necessary to explain the origin of the secondary tumours, is to be compared to a spermatic influence produced in some unknown manner by the parent tumour. But there is also a histological reason in support of this analogy.

Vacuolation of cells compared to the first changes in the ovum after fertilisation.

It is not altogether fanciful to trace a similarity between the changes that the ovum undergoes after fertilization and the changes in a cell, such as a liver cell, undergoing vacuolation; and such a comparison would probably appear less fanciful if the first changes produced by impregnation of the ovum were better understood. It is generally agreed, among much that is doubtful, that in the ovum, after fertiliza-

tion, the nucleus disappears,—whether a complete disappearance or a change to a position where it cannot be traced, is uncertain,—and that in the place of it there appears a vacuole or cavity (nucleus cavity). These changes, whatever they may signify, are the direct and earliest effects of fertilization. Now the process of vacuolation may be described in similar terms: the nucleus, as such, disappears, and a cavity, which may, however, be very much larger than the nucleus, appears in its place. There is to be added also, as regards vacuolation, that what was the nucleus, or at least the substance of it, reappears either at one pole of the cell or as a round mass lying free in the cavity. Although the parallel cannot be continued far, there is similarity enough in the manner of transformation in both cases to make it probable that the cause is similar. Thus we have additional evidence that the extraneous influence in the production of secondary tumours is comparable to a spermatic influence.

Occurrence of vacuolation in other pathological conditions;

The process of vacuolation that has been described above as concerned in the formation of tumour cells, occurs in other pathological conditions, and there are good reasons for considering an important class of cell changes in the normal organism to be a process of the same nature. Setting aside the occurrence of the characteristic vacuolated cell among the products of inflammation of the synovial membranes of joints, and of other serous membranes, we shall limit our attention to the occurrence of the vacuolation process in the epithelium of mucous membranes. Vacuolated cells were described by Remak as occurring among the products of catarrh of the respiratory mucous membrane, and this observation has been made familiar by subsequent descriptions. In this case there is no doubt of the nature of the phenomenon, but other instances that are about to be adduced as exemplifying the same process are not equally admitted. Many of the cells found in colostrum have a greater or less resemblance to the forms of vacuolated cells described above. Thus Langer describes cells containing a vesicle filled by a fat globule and having a crescentic mass of protoplasm disposed on one side.* The epithelial cells of the testis employed in the formation of spermatozoa frequently exhibit the characteristic appearances of vacuolation, and La Valette describes as occurring in the seminal fluid shapeless masses of protoplasm, which he identifies with the corresponding masses found in colostrum. They are said by him to be probably "unverbrauchte Protoplasmareste," as it were, waste products of the epithelium produced along with the spermatozoa.† Again, the salivary and mucous corpuscles are cellular bodies formed from the epithelium along with the proper secretion, and whether they occur only in a catarrhal state of the gland or in the normal condition is unessential to the present argument. The peculiar cup-shaped appearance of many goblet cells, depending on a crescentic mass of protoplasm at their attached border, and prolongation of the same on each side of the cell, has a real resemblance to a vacuolated cell. The crescentic mass and the lateral prolongations of it are distinguished by their property of deep staining in exactly the same way as the peripheral mass in a vacuolated cell.

and under normal conditions.

It is contended that in the above instances there is a resemblance, as regards the form of the cells, to the product of vacuolation previously described, and the following considerations are brought forward in proof of an identity in the mode of their production.

In the production of secretion from mucous surfaces (that is to say,

* Stricker's Handbook, p. 632.

† Stricker's Handbook, Art. The Testicle.

in all cases except the parenchymatous organs, the liver and kidney), the secretion is formed if not entirely at least in a great measure at the expense of the individual epithelial cells. This is most obvious in the case of the mammary and sebaceous glands, but although it has not been clearly proved for all other cases, there is no doubt that it is the general law for the epithelium of surfaces. This law is expressed in the following terms in MM. Cornil and Ranvier's Manual of Pathological Histology :*

"This evolution is the leading physiological fact of all epithelium : all "their elements are transitory, being born, coming to maturity, and "dying within a variable space of time. . . . It is a well-proved "fact that, in glands, the epithelial cells are shed to give exit to the "excreted substances, and that their own contents are an essential part "of the secretion. . . . Glandular epithelium, of which the cells "may be of the pavement variety, or cylindrical, pyramidal, &c., is the "subject of a constant evolution. Thus it is that in the glands of the "stomach one sees the cells that are at first cylindrical becoming "spherical from distending themselves with juice, falling into the "lumen of the gland, aud getting destroyed in the act of giving up "their contents. So the cells of colostrum are no other than the cells "of the mammary acini, and although in the milk they are no longer to "be found, it is because the cells are destroyed in setting free the fat "which they contain."

The vacuolation process in secreting epithelium.

Now the occasional occurrence of protoplasmic masses in association with the fluid products of secreting cells has an important significance in respect of the essential nature of the secretory process. We are, in fact, entitled to argue that there is potentially present in every secreting cell, both a fluid or vesicular and a cellular product, and that there is a co-relation between those two. Where the substance of the epithelial cell is most completely devoted to the formation of the secretion, the solid product is reduced to a minimum, and in those cases where the solid product of the secretory cell, so to speak, asserts itself, the secretion loses accordingly. The best example of this is the colostrum or half-formed secretion of the mammary gland, where the mammary epithelium, instead of being entirely transformed into milk drops, undergoes only a partial transformation, leaving a certain amount of the protoplasm as an accessory product. The catarrhal cells of the bronchi are probably to be explained on the same principle. Salivary and mucous corpuscles, if indeed they also are not due to a catarrhal condition of the mucous surface, are to be regarded as instances where the cellular elements in the secretion have no pathological import. The crescentic masses that form the base of goblet cells, and the protoplasmic bodies found in the seminal secretion are of sufficiently common occurrence to be regarded as normal. The spermatozoa themselves, as well as the ova, may be adduced as instances of solid products formed in the vacuoles of epithelial cells, and if this interpretation of the seminal and ovarian secretions be correct, we are here furnished with an instance where, in the secretion of epithelial cells, the solid product becomes the essential, and the fluid the accessory.

Secretion a process of endogenous cell formation.

The conclusion, then, that we arrive at from the above considerations is, that the process of secretion in the epithelial cells of mucous surfaces is essentially a process of endogenous cell-formation.

The account usually given of the production of secretion in the

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* Manuel d'Histologie Pathologique, p. 29.

epithelial cells is merely pictorial; a passage already quoted will serve as an example:

"C'est ainsi que dans les glandes de l'estomac on voit des cellules "primitivement cylindriques devenir sphériques en se gorgeant de "sucs, tomber dans la lumière de la glande et se détruire en laissant "échapper leur contenu."

As so expressed, the life of the secreting cell is nothing else than a chapter of accidents, a succession of events apparently arbitrary in their occurrence. If, however, the law of endogenous cell-formation be applied to this particular form of cellular activity, the various changes in the cell find a rational explanation. The most valid objection to such an explanation will be found to be directed against the terms employed. It is true that in most of the cases that are here referred to the so-called process of endogenous cell-formation, no cells are formed; but that is a paradox which depends on the name having been adapted to only a part of a more widely-spread phenomenon. It may be instructive again to contrast the endogenous mode of cell formation with the mode by division. What distinguishes the former, is the collateral production of a vesicle or vacuole containing a fluid, the result of a transformation of the cell protoplasm. In the latter form of cellular activity, the cell divides into several pieces, each of which becomes exactly like the original cell. One might say that in the endogenous mode there is a provision for a fluid product, and it is according to many analogies in nature of the adaptation of a general plan to particular ends, that such provision may be magnified into the essential feature of the process.

In searching for a confirmation of the above hypothesis as to the essential nature of the process in secreting epethelium, the writer has found that a similar view was entertained by a zoologist, Friedrich Will, who is quoted by Professor Virchow in 1851. In making an investigation of the seminal secretion throughout the animal kingdom, Will arrived at the conclusion (as stated by Virchow) that "all secretions proper are the "result of a process of cell formation, and, indeed, of endogenous cell "formation (*durch Zellenbildung, und zwar durch endogene Zellenbil-"dung vermittelt*)." Virchow adds, "It is to be hoped that the propo-"sition, thus broadly stated, is to be understood as applying only to "invertebrate animals; but it seems, at least, to indicate the wide "distribution of a phenomenon that has, perhaps, a greater significance "in physiology, and which may lead us, in course of time, to regard "the process of secretion from a higher and more general point of "view."* The theory of Will has not passed into the text-books of physiology, and appears, indeed, to have been lost sight of.

Theory of the malignancy of primary epithelial tumours.

The hypothesis that the secretory function of mucous membranes is performed by means of a process in the epithelial cells identical with the process that is known, under other circumstances, as endogenous cell-formation, has a very great significance as regards the malignant tumours of epithelial parts. What this significance is may be illustrated in the case of the mammary gland.

Illustrated in the case of the breast.

The colostrum corpuscles that are found in the milk at the commencement of the secretion, and which are observed more perfectly in the acini of glands that have lately ceased to secrete, may be taken as a starting point. They are the epithelial cells that have been shed in a half-transformed state. Their activity is still the functional activity, as contra-distinguished from the plastic; but, owing, no doubt, to an immature or subsiding functional stimulus, their transformation into the fluid of the secretion is incomplete. The fluid contained in their

* Archiv. III., 224.

vacuoles is all that can be called secretion in the proper sense of the term, and from the same point of view the associated mass of protoplasm has a purely negative importance, and is to be viewed as so much waste. But if the epithelial cell is regarded, not as performing a function, but as obeying the law of endogenous cell-formation, it is obvious that the fluid in the vacuole and the solid mass on one side of it are co-ordinate products, each of which is more or less important according to its degree of predominance over the other. Under this correlation, the vacuolation may be extreme and the cellular part insignificant, or *vice versâ*, according to circumstances ; and among colostrum cells many gradations are in fact observed. If we consider the complete transformation of the epithelial cell into a fluid substance, as in the normal secretion of milk, to be the case where the vacuolation reaches its extreme limit, we may also conceive of the other extreme in which the solid or cellular element preponderates ; and the latter, though in the particular circumstances not a useful product, may be spoken of as also a product of the secretory process. Now when one calls to mind the special characteristic of cells produced endogenously, the bearing of these points on the question of malignancy will become apparent. It is the characteristic of the products of endogenous cell-formation that they assume a type different from that of their parent cell. As it has been already expressed, the mode of cell-formation by division is the plastic or hyperplastic process, while the endogenous mode is metaplastic. Metaplastic, again, is in pathological language equivalent to heteroplastic. In this manner, then, in the exercise of the glandular function, and by virtue of the law according to which the function is performed, there would be produced, on occasion, from the epithelium of the gland, elements that were not themselves true epithelium, and that were in fact heteroplastic.

Now, continuing the illustration of the mammary gland, there is abundant reason to suppose that in cancer of the breast the epithelium is the point of departure for the new growth. This view is held by many pathologists, notably by Professor Waldeyer, who describes cancerous tumours of the breast (and of other epithelial situations) as a growth of atypical epithelium. But it is precisely the atypical element in the new growth that remains unexplained. There is nothing in the explanation of the histogenesis of the growth given by Professor Waldeyer to show wherein it differs from a mere hyperplasia. Other pathologists, again, such as Professor Virchow, have been led, in the absence of such an explanation, to assign the origin of the epithelial-like cells of the tumour to an "epithelial infection"* of the connective tissue, rejecting, as it were, the strong evidence of epithelial origin for the sake of being able to account for the heteroplasia of the growth. Thus, Virchow, after stating the epithelial theory, and the explanation of the apparent exceptions to it, adds significantly, "Ich meinerseits bin durch diese Ausführ ungen nicht überzeugt; ich halte an der primären Heteroplasie aller Krebse fest."† Now, if it can be shown that the cellular product of secreting epithelium is no longer epithelium, but a heteroplastic product, and that by virtue of the law of endogenous cell-formation, according to which the function is performed, such an objection as is here stated is no longer tenable.

The mammary gland has been taken as an illustration, and it is obvious that the sebaceous glands, and the *rete mucosum* in general

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* The expression is used by Klebs. The theory has been most explicitly stated by Virchow and W. Müller.

† Cell. Path. 4th Edition, p. 469–70.

together with the epithelium of the digestive and genito-urinary tracts, are subject to the same process of reasoning. The attempt would therefore be made to associate the origin of primary tumours of those parts with irregularity in the performance of the function of the part, and to explain the malignancy of tumours so arising.

With this view an investigation was made of a series of mammary tumours. They were 21 in number, 8 of them from the human subject, 11 from the dog, and 2 from the cat. It became apparent, however, in the course of the investigation, that there was another factor, special to the mammary gland, which played a part in the formation of new growths, viz., the periodical evolution and involution of the gland. The information hitherto acquired as to those remarkable changes occurring normally in the mamma, proved unfortunately to be very scanty, and it became necessary to make an original investigation of the subject. This collateral inquiry is not yet (March 1874) far advanced, and a report on the cases of mammary tumours is therefore postponed.

No. 5.

Researches on the Chemical Constitution of the Brain.

By J. L. W. Thudichum, M.D.

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I.—METHOD PURSUED FOR THE ISOLATION OF IMMEDIATE PRINCIPLES.

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Material selected.

Anyone who would proceed to an extensive chemical investigation of the brain finds it impossible under ordinary circumstances to procure, in the perfectly fresh state, and with the perfect freedom from disease which are indispensable, such a supply of material from the human subject as will even approximately suffice for his purpose. Therefore, as material

Weight of ox brains.

Preparation of brains

for the present very large inquiry, ox brains* have in most part been preferred; and of these more than a thousand have been used. Five brains weigh on an average 1,780 grms., or eight weigh six pounds. They are washed once and freed from clotted blood. They are next carefully skinned, the arachnoid and pia mater being removed by means of fine anatomical forceps. When the brains are entire they may be skinned in the ordinary anatomical manner, which employs two pairs of forceps, worked simultaneously and antagonistically by both hands of the operator. When, however, the brains are much broken up, each piece must be held in one hand while being freed from membranes with the other. The skinned parts are again rinsed in water, and then placed in water for a short time; next placed upon a sieve to drain, and then submerged in methylated alcohol of 85 °/₀ by weight in volume strength, previously purified by distillation over tartaric acid. Great care is necessary to supply a sufficient amount of alcohol, so that the brains may be quickly dehydrated and hardened. For if the alcohol is too dilute, or becomes too dilute by being insufficient in quantity, the brains remain soft and unworkable, and decompose with a fetid odour. For the same reason all brains, before submersion in alcohol, must be broken up, or sliced into small pieces, so that they can be easily penetrated by the alcohol. This is required even when strong alcohol is used, as this is liable to harden the outer shell merely, and leave the inside of the brain to soften and decompose.

Submersion in alcohol.

First extracts.

The washing and submersion in water probably remove small quantities of extractives and soluble salts, besides the blood, and must therefore be carried out with care. The solutions so obtained are thrown away, but the alcoholic solutions in which the brains have been hardened are purified from albumen by boiling and filtration, freed from spirit by distillation, and evaporated to the consistence of extracts on the water bath. They are to be considered as water-extracts, and are mostly free from specific brain substances, and only yield extractives and salts and other matters, which will be described in the relative chapters.

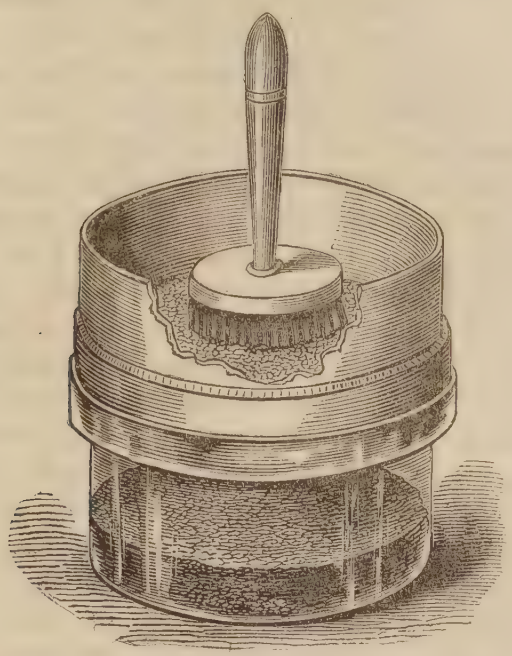
Mincing the brain.

When the brains are well hardened in the alcohol, which frequently requires the repeated removal of the watery spirit and substitution of fresh strong spirit, they are passed through a rotary mincing machine, and the minced portions are again mixed up with strong alcohol. This pulp is now worked through a very fine hair sieve, having 144 meshes to the square inch, and each of the twelve strands of hair crossing the square inch in one direction, being composed of eight single horse hairs; the sieve stands upon a glass-receiver, a so-called fern glass, in such a manner that it cannot move, and no matter which passes the sieve can be lost. The trituration on the sieve is effected by a strong circular furniture brush, which is incessantly rotated over the sieve by the hands of a workman. When passed through the sieve

Comminution through hair sieve.

* The ox brains as obtained from the butcher are generally much suffused with blood. This arises from the mode in which the animals are slaughtered; a blow is inflicted upon the frontal region with the pole-axe, whereby a piece of skull is punched in and pressed into the brain. Immediate unconsciousness and paralysis is the result. In addition to this the slaughterer destroys the spinal reflex-centres by introducing a cane through the hole in the skull into the spinal canal and destroying the marrow by stirring it about. The skull is afterwards cloven, and the brain taken out in two halves. Where animals are killed by the Jewish method of cutting a gash across the throat and bleeding the animal to death, the brain may be removed uninjured, and may even be washed *in situ* by injecting water through the *carotids*. But this proceeding is laborious, and offers no advantages as regards the chemical examination of the brain. For investigations of the spinal marrow, however, animals so killed are alone suitable, as by the pole-axe and cane method the spinal marrow is almost entirely destroyed.

the brain is in the state of a very fine pulp or purrée, and is ready for extraction. All other modes of comminution which have been



Sieve on Fern-Glass and Rubbing Brush for Comminuting Brains.

recommended are less useful than the foregoing; they are either inefficient or slow and laborious; in particular trituration in a mortar with a pestle is very inefficient. All methods of comminution which do not reduce the brain to the finest possible pulp, or smooth paste, must be rejected, as from imperfectly comminuted brains the immediate principles are necessarily most imperfectly extracted.

Bad modes of comminution.

The smooth paste of brain matter is now mixed with a considerable amount of alcohol of 85 %, and heated in a well-tinned large saucepan over a gas lamp by a star-burner or any other heat source, while being stirred with a wooden rod without intermission. When it has reached the temperature of 45° the mixture is removed, and immediately poured on a filtering cloth stretched and tied over the top of a large earthenware jar or pan. The filter is covered with a wooden cover. When the liquid has percolated, the pulp is removed from the cloth with a flat spoon, and again placed in the saucepan, mixed with spirit, heated to 45° while being stirred, and again placed upon the same filter as before. This operation is repeated in all about five times, when the brain matter is exhausted of all matters which alcohol will dissolve. The matter is now tied up in the cloth and pressed in a screw press. It comes out as a solid, somewhat elastic cake of *albuminous* or *insoluble residue*, of which the analysis and description will be given in a future chapter. I have always arrested the heating at 45°, because it has been so frequently stated that when brain matters are heated beyond they decompose. But since I know the behaviour of the isolated brain matters, I have great doubts about the correctness of this statement, and should in a given case think it quite safe to boil the mixture. But the low heat is certainly efficient and convenient, and need not therefore be overstepped. For the same reason a saucepan of cast-iron of about three gallons capacity is preferable to any other vessel of tin or glass. The earthenware jars or pans should be of a capacity of from 12 to 15 gallons. I have found them most useful, and carried on all operations from the soaking and hardening to the last filtration of the buttery precipitate with their aid.

Extraction with warm alcohol.

Vessels used.

Insoluble albuminous residue.

Degree of heat employed.

Advantages of certain vessels.

The alcoholic extracts are all united, and allowed to cool during from 12 to 24 hours. In hot weather the cooling must be assisted by placing

Treatment of alcoholic extract.

The precipitate.

Filtration.

Pressing.

White matter.

Substances contained in white matter.

Keeping qualities of white matter.

Alcoholic filtrate distilled.

Buttery matter.

Substances contained in buttery matter.

Alcoholic filtrate from buttery.

the jars into a tub and surrounding it with cold water and ice. Two such tubs are conveniently produced by cutting a port wine pipe into two equal halves. The extracts during cooling deposit a large amount of *white crystalline and granular precipitate*, which adheres to the sides and covers the bottom of the vessel, while the alcohol is perfectly clear, though coloured slightly yellow. The whole is filtered through a cloth stretched over a pan, and when the entire precipitate is collected on the cloth, and has been condensed by stirring with a spoon, the cloth is removed, tied up, and placed into the screw-press, and all mother-liquor thus removed. When taken out of the cloth the precipitate presents itself as a hard white cake, which can be broken into pieces, and constitutes the particular white matter of Vauquelin, and will in the following always be signalised as *white matter*. When the abreviations *w. m.* occur in the description of any preparation, they indicate that the preparation has been extracted from this white matter. I shall not describe this white matter any further, nor have I instituted any experiments upon it such as Vauquelin made, because it is evidently a very complicated mixture, containing nearly the whole of the substances to be described as the cerebrines, stearoconotes, cholesterines, kephalines, myelines, and lecithines, and small quantities of other matters. The processes by which these substances may be extracted will be given lower down, after the description of the treatment of the alcoholic extract has been completed. Here it may yet be stated that the white matter can be preserved in stoppered bottles in a cool place and protected from light almost unchanged for a very long time. In contact with absolute alcohol it also remains unchanged, though graduâlly yielding a yellow extract; but in contact with ether it yields kephaline to the latter, which is quickly oxydised into a red substance having a green fluorescence; this effect seems due to the peroxyde of hydrogen produced during the oxydation of the ether. I have therefore limited the use of ether to the most necessary operations, and then cause the substances to pass through these with great despatch, so that this oxydising effect of the ether is as far as possible avoided.

The Alcoholic Filtrate from the White Matter is now placed in a capacious tinned copper still, and a great part of the alcohol is distilled off. When a certain degree of concentration has been obtained, which is determined by experience, the hot liquid is thrown into a pan, and again allowed to cool, assisted if necessary with cold water or ice. It now deposits a second quantity of matter, which is less solid and more coloured than the first and after filtration remains on the cloth as a semi-solid plastic substance, to which I have given the name of the *buttery matter*. This can only be freed from mother liquor by manipulation with a spoon, and must not be pressed too hard, as it is liable to pass through the meshes of the cloth. The buttery matter consists of much cholesterine, lecithine, little myeline, kephaloidine, and some cerebrine, and small quantities of other matters; the substances are therefore qualitatively mainly the same as in the white matter, but they are present in entirely different proportions. The buttery matter also keeps well in a bottle by itself, or in the presence of alcohol, but should also not be kept long in ether.

The Alcoholic Filtrate from the Buttery is again distilled so long as good spirit passes over and no precipitate ensues in the fluid. When these conditions are exhausted it is placed in a large dish on a water bath and evaporated. At a certain period *oily drops* make their appearance, which adhere to the sides or float in the fluid, and unite to larger round globular masses. They separate easily while the fluid is

hot, but when the fluid cools they swell, become flaky and distributed in the fluid and cannot be filtered. They are best separated while hot by a separating funnel, to which they adhere, while the fluid sinks down ; or they may be collected on a paper filter on a hot funnel. This matter has received in my laboratory the title of the LAST OILY, by which it will be signalised in this essay. It consists mainly of phosphorised bodies with little cholesterine, and some peculiar not yet accurately defined matters, amongst them oleate and margarate of ethyle.

Last oily matter.

The Filtrate from last Oily constitutes the ultimate mother liquor and contains all matters from the brain which are highly soluble in water, such as the salts, the extractives, and soluble immediate principles to be described. This liquid is evaporated on the water bath to the consistence of a thin extractive and placed in bottles until further examined as will be described. While the preparation accumulates it is well to keep the extract covered with some absolute alcohol to prevent the formation of mould on its surface.

Filtrate from last oily, last mother liquor.

Treatment of the White Matter.—The white matter is now to be extracted with cold absolute alcohol. The alcohol must be cold to leave as much as possible of the cerebrine, cholesterine, kephaline, and myeline undissolved, and to dissolve all the lecithine; and it must be absolute so that the cerebrine and kephaline may not become hydrated in any way, but rather more dry under the influence of the alcohol. The best mode of mixing the alcohol and white matter intimately is by the hands in a wide earthenware pan. While the workman is kneading this mixture no light or fire is permitted in the laboratory. The extraction is so regulated that the same alcohol is used to extract several portions of white matter in succession, so that most of the alcohol employed comes away nearly saturated with what it can dissolve. As the extraction proceeds the white matter becomes plastic and yields but little to alcohol, and then the extraction is interrupted, the residue thrown on a cloth, drained and dried.

Treatment of white matter.

Mixing with the hands.

Treatment of the Alcoholic Solution.—The alcoholic solution is first exposed to frost, when mostly a white deposit of myeline is observed, which may be filtered off on a funnel surrounded with freezing mixture. The alcoholic filtrate is then reduced in bulk by distillation, and again exposed to frost, and this treatment is repeated. In this manner several deposits are obtained, which become more and more fluid and oily, until at last the solution is a thick oil, which when exposed to the air dries out to a plastic waxy mass of a neutral ether mixed with much cholesterine and little kephaline and myeline, and lecithine.

Exposure to frost.

Myeline deposited.

From the successive deposits some myeline may now and then be obtained in a crystalline form, by resolution in alcohol, refreezing, washing with ether, but by solvents alone the bulks of these matters cannot be separated. It is therefore best to treat the alcoholic solution as follows :—

Isolation of small deposits of myeline.

Platinum Chloride Treatment of the Alcoholic Solution.—To the alcoholic solution freshly prepared pure absolute alcoholic solution of platinic chloride is added as long as a precipitate is thereby produced. The voluminous yellow precipitate is thrown upon a filter, washed with absolute alcohol, removed from the filter into a bottle, and shaken with absolute alcohol, again placed on a filter, drained upon a cloth and without being allowed to become dry or waxy treated as follows :—

$Pt\,Cl_4$ Treatment.

Treatment of the Platinic Chloride Precipitate.—It is placed in a large stoppered bottle, mixed with a large volume of ether, and shaken from time to time during several hours, until there are no lumps perceived in the bottom of the bottle, and the contents consist of a fine yellow powder and an orange-coloured solution only. Then powder and solu-

Treatment of $Pt\,Cl_4$ precipitate with ether.

Insoluble in ether salt myeline Pt Cl⁴.

Treatment of ether solutions and washings.

tion are separated by filtration. The salt on the filter has to be repeatedly removed from the filter, to be shaken with ether in a bottle, and to be refiltered until pure; washing on the filter alone is almost impracticable. The insoluble salt constitutes *myeline platinic chloride*, with its modifications to be described lower down. It easily dries in air to a yellow somewhat waxy mass, which is easily powdered. The ether solutions and washings are united, concentrated to a small bulk by distillation from the water bath, and then precipitated by the addition of much absolute alcohol. The precipitate is washed on the filter, also by shaking in a bottle with alcohol, again filtered, drained on blotting paper, and placed on porcelain or glass dishes, in a vacuum over sulphuric acid to dry.

Soluble in ether salt and Pt Cl⁴.

This soluble in ether salt constitutes probably *lecithin platinic chloride* with its modifications to be described lower down. It contains, however, some kephaline, from which it has to be freed by a special process to be described under the chapter which treats of the purification of this precipitate.

Treatment of alcoholic mother liquor

Alcoholic Filtrates from the Platinic Chloride Precipitates.—These are coloured yellow, and on standing deposit cholesterine in crystals, and a trifling quantity of platinic compounds. They are filtered, concentrated and treated with hydrothion and water. The water removes *chlorides*, which may be investigated as regards the bases. The platinic sulphide mostly retains *organic matter*, which must be extracted by alcohol and ether, applied cold and boiling. Besides residues of the principal matters already extracted they contain *peculiar neutral bodies* in small quantities, which are not fats commonly known, perhaps oleate and margarate of ethyle, and require a more elaborate study hereafter. Neutral fats so called, trimargarine, tristearine, tripalmitine, trioleine, if at all present in brain substance, are so in extremely small, hardly appreciable quantities.

Peculiar neutral bodies, not fats

Treatment of the White Matter from which Lecithine and some Myeline has been extracted by cold absolute Alcohol.

Extraction with ether.

This matter is to be extracted with ether. For this purpose it must again be kneaded in a pan, this time with ether, by the hands of workmen. All shaking in bottles, trituration in mortars, is perfectly futile. With operations in ether the presence of light or fire in the laboratory is still more to be avoided than with operations in alcohol. The extraction with ether causes the previously plastic white matter to assume a grumous pulverulent consistency, and to yield a matter to the ether, which quickly becomes red.

Difficult problem.

Kephaline.—The filtration of the solution from the fine white insoluble matter is one of the greatest difficulties of the whole series of operations. I find the following process the most effectual. The mixture is thrown on a cloth in a funnel surrounded with freezing mixture, and as much liquid is allowed to percolate as may. When the cloth is full and ceases to filter it is tied up, and with the pasty mass placed under a screw press.

Pressing.

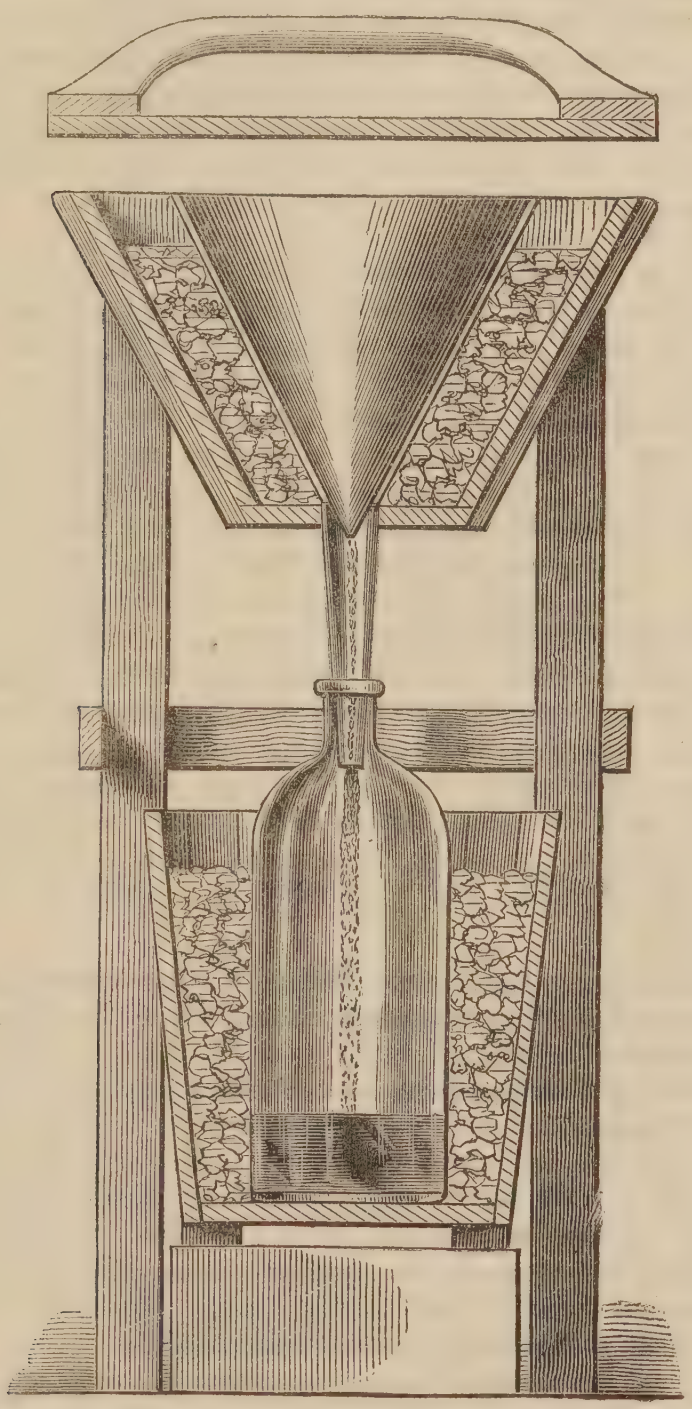
The white milky matter now squirts in all directions, unless the press is worked very slowly, and a constant current of pure ether is directed upon the outside of the cloth. In this manner, with much labour, requiring the constant attention of three operators, the matters are at last separated into a hard residue of *cerebrines* and *myelines*, and into a solution containing *all cholesterine*, *all kephaline*, and *some myeline*.

Separation of kephaline from cerebrines, &c.

Treatment of the solid Residue for the Separation of the Cerebrines from Myeline.—These residues when dry are intermediate between wax

and gum arabic in appearance. They consist in the main of the cerebrines, with the stearoconotes, and some myeline. The latter has to be extracted with hot ether, in which the two former are quite insoluble. The extraction has to be continued until the residual matter of cerebrine, &c., *is free from phosphorus.* This requires large quantities of ether, as it quickly evaporates during filtration on a funnel surrounded by warm water. The receiving bottle stands in a pail surrounded with ice, so that the hot ether is immediately cooled. It immediately deposits the myeline as a white powder, which is afterwards separated by filtration on the funnel surrounded with freezing

Extraction of myeline by hot ether.



Filtering Stand of Wood with Funnel which may be surrounded with Warm water or freezing Mixture.

mixture. When the funnel is not surrounded with freezing mixture, much myeline re-dissolves in the warmer ether. This portion is only obtained by distillation of the ether, and is not so pure as the portion deposited from the ether on cooling. The myeline is purified further as will be described under the chapter relating to it, and the cerebrines are

Distillation of myeline solution.

separated from stearoconote and from each other, and from any remaining myeline as will be described under the headings relating to them. The myeline may also be extracted with cold pure benzole, in which the nitrogenised bodies are quite insoluble.

Freezing out cholesterine and myeline.

Treatment of the Ether Solution containing Kephaline and Cholesterine, separated by Filtration from the Cerebrine, &c.—This solution is colored red and fluoresces green. It is concentrated by distillation from the water bath, placed in stoppered bottles, and exposed to frost in an ice and salt mixture. If concentrated it becomes solid with crystals of cholesterine and granules of myeline ; in this case it must be thawed until pulpy, filtered on the funnel surrounded with ice, and the filtrate again frozen. In any case the *solution must be frozen and filtered cold until it remains quite clear, even in the sharpest freezing mixture.* In that case most of the kephaline remains in solution, and retains only little cholesterine and very little myeline. The cholesterine which crystallises out, contains myeline and little kephaline, from both of which it may be separated as will be described under the chapter relating to the purification of cholesterine.

Precipitation of kephaline by alcohol.

The ether solution containing the kephaline is now mixed with twice its bulk, or as much as may be required to effect complete precipitation, of absolute alcohol; a voluminous, whitish, flaky, adhesive precipitate ensues, which becomes darker as it condenses; this is *crude kephaline.* The mother liquor when clear after some standing, is decanted, the kephaline is rinsed with alcohol, collected, manipulated with a glass rod or spatula so as to remove alcohol enclosed in its meshes, spread on a glass dish and dried in vacuo, to be purified as will be described under the chapter relating to kephaline.

Precipitation of matters by cadmic chloride.

The ether alcohol mother liquor is treated with an alcoholic solution of cadmic chloride, whereby a mixture of compounds of kephaline and myeline with this salt falls down, which is collected, washed, and further purified, decomposed and identified as will be described. The mother liquor from these cadmium salts is distilled to dryness, treated with water for the removal of cadmic chloride, and the insoluble part is again boiled with small quantities of alcohol,

Cholesterine and neutral peculiar matters.

when cholesterine is obtained in crystals, and a quantity of neutral matter, part of which is oily, part solid; both will be described in a separate chapter.

Ether process applied to white matter

Another Process for separating White Matter into its Constituents.—In many of my earlier investigations I used the following process which for the purpose of abbreviation I will term " ether process, W.M." This yields the cerebrines very quickly and directly, and leaves little myeline with the cerebrines. It also yields kephaline, but in lesser quantity as much remains in the mother liquors. But the myeline, lecithine, and cholesterine remain in the ultimate mother liquor, and can be separated only by cadmic or platinic chloride. The precipitates obtained are more complicated than in the first process. But under given conditions the process possesses advantages and is therefore at all events here described as an alternative second in value.

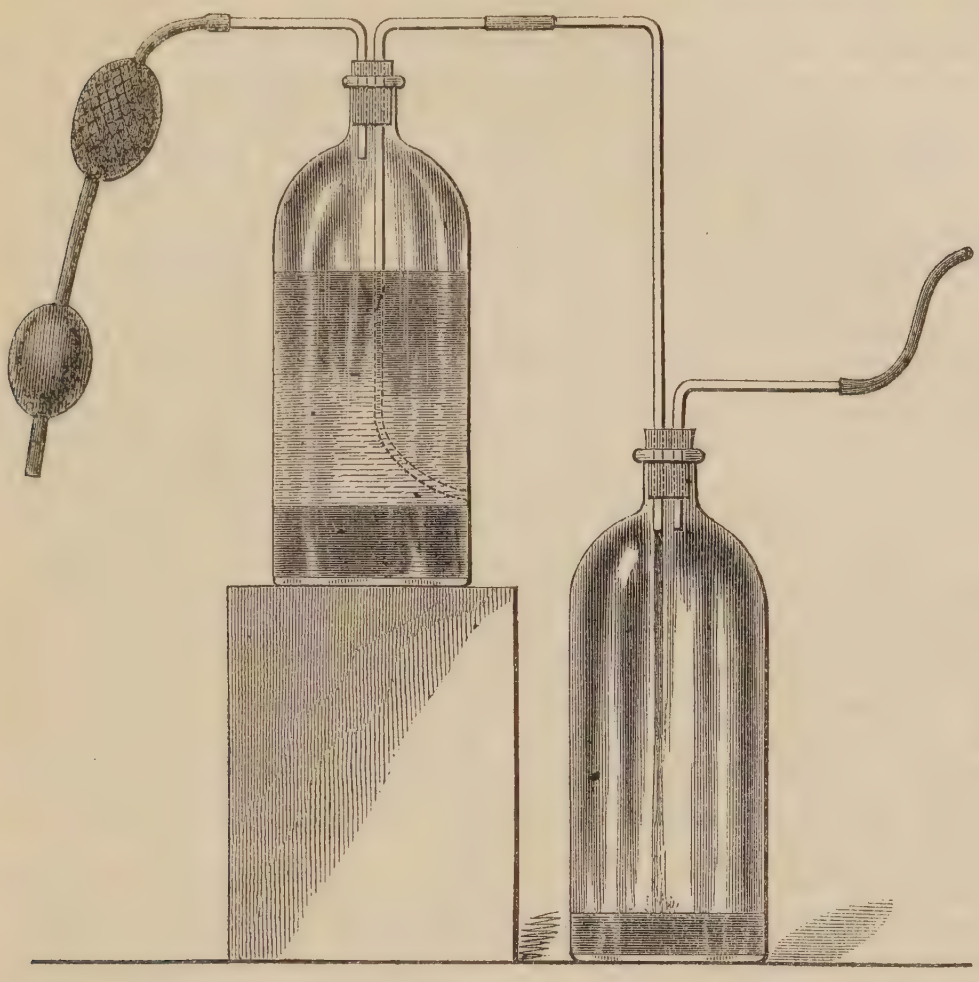
Extraction of W. M. with ether.

The white matter is fully extracted with ether in stoppered bottles, with the precaution of using the same ether for several portions of white matter so as to obtain saturated solutions. All secondary solutions and washings are concentrated by the still. The whole of the solutions are now exposed in bottles (stoppered) to a strong freezing mixture of ice and salt, and the clear coloured ether is quickly siphoned

Description of siphons.

off the dense white deposit. All siphons are of glass tube, with movable caoutchouc joints, and mounted in corks, so that they can be applied to the bottle to be emptied on one side and to the bottle to be filled on the

other side, air tight, and be started either by blowing (with the air bellows) or by suction at the opposite end. The inner limb of the



Arrangement of Siphon for decanting by Pressure and Suction.

siphon is so curved as to be near the side of the bottle and easily visible, its point is directed sideways to prevent an upward rush of deposited matter.

This apparatus gives to the operator full power to start and arrest the flow of the ether whenever he finds it desirable, and enables him to regulate the suction pipe, and so take off the last portions of ether above the deposit, without losing much ether or being molested by it. When the principal mother liquor is removed, pure ether is thrown upon the deposit which is again frozen. The deposit is much less soluble in pure ether than in the mixture of dissolved matters, a peculiarity shewn by almost all brain substances. The deposit, when dense, frequently forms a firm cake at the bottom of the bottle, which comes off as a round disk. These "*first deposits by frost from ether extracts of white matter*" are separated into cerebrine and myeline, as will be described lower down. Perhaps the most massive and substantial quantities of myeline can be obtained from them, that can be obtained at all without the circuitous route of combination with metallic salts.

Cerebrine and myeline deposited by frost.

When the ether extracts give no further deposits on exposure to renewed freezing mixtures, they are treated with absolute alcohol until all *kephaline* is precipitated. If the alcohol be watery, even slightly, say of 80 to 90 °/₀ strength, the deposit contains much cholesterine, particularly if the ether solution is concentrated. Absolute alcohol should therefore be always taken as well as absolute ether for these operations; many other reasons which will appear in the sequel support this desideratum to the extent of making it an absolute condition of perfect success.

Precipitation by alcohol of kephaline, &c.

Absolute ether and alcohol required pure.

Both ether and alcohol should be pure, *i.e.*, not made from methylated spirit, but from pure spirit, and always be specially tested for purity and strength.

Precipitation of mother liquor with cadmic chloride.

The kephaline so precipitated is much less pure than that obtained in the first process, and requires, therefore, more purification by the processes to be described. The ether-alcohol mother liquor is now treated with cadmic chloride dissolved in alcohol, so long as a *precipitate* takes place. The precipitate is filtered off on a cloth, pressed hard, and immediately placed in ether. A cadmium salt dissolves, and will be described in the following as "soluble in ether cadmium salt from white matter after kephaline." It consists of kephaline with another phosphorised matter combined with cadmic chloride. Another salt remains insoluble in ether, and will be described in the following as "insoluble in ether cadmium salt from white matter after kephaline." It consists mainly of myeline combined with cadmic chloride. The immediate principles can be obtained from these cadmium precipitates only with difficulty and by laborious and long processes, and therefore they are less useful, if applied at this stage of the operation, than might at first sight be expected; but they enable the operator to completely extract from the phosphorised substances the cholesterine which remains in the mother liquors. The mother liquors are distilled in the water bath until all ether is removed. On cooling they then deposit cholesterine in crystals, nearly pure. After further evaporation they deposit more cholesterine, mixed with some cadmium salt; ultimately they leave a mixture of cholesterine and neutral peculiar matters, to be isolated and purified as will be described.

Two salts, one soluble the other insoluble in ether.

Treatment of ether alcohol mother liquor.

Buttery treated with alcohol.

Treatment of the Buttery Matter.—The buttery matter is kneaded with absolute alcohol in a pan, and allowed to extract during 24 hours. The alcohol is then renewed and the kneading repeated frequently until the undissolved matter appears as an adhesive, semi-solid mobile substance, yielding little or nothing to alcohol. This insoluble matter is mainly *kephaloidine*, with *cholesterine*, little *myeline*, and less *cerebrine*. It is treated with absolute ether, in which kephaloidine easily dissolves; myeline dissolves a little, leaving the bulk of myeline and cerebrine as a white undissolved mass. The latter are filtered off and washed. The ether solution is exposed to a freezing mixture until it remains permanently clear after filtration and renewed exposure. It is then mixed with sufficient absolute alcohol to precipitate all matters which will go down. When the mixture is clear the ether alcohol is decanted, the precipitate collected, rinsed with alcohol, spread on glass dishes, and exposed in a vacuum to dry. It constitutes *kephaloidine*, which must be purified, as will be described under the chapter relating to this substance. The ether alcohol mother liquor of kephaloidine is treated with platinic chloride, or cadmic chloride, and the relative precipitates are separated by ether, so as to yield soluble in ether salt (kephaloidine and lecithine salt), and insoluble in ether salt (myeline salt). These salts must be further treated like those obtained from white matter. They are small in quantity.

Insoluble matters separated and treated with ether.

Kephaloidine isolated.

Alcohol extract of buttery.

The alcoholic extract of the buttery matter can be treated by gradual concentration and exposure to freezing mixtures, when myeline and cholesterine, ultimately cholesterine alone, are deposited; but the process offers no advantage except preliminary information, as each fraction of deposit has to be treated in the same manner in which I now treat the entire solution. It is mixed with as much solution in absolute alcohol of pure platinic chloride as will effect complete precipitation. The voluminous precipitate is filtered and washed with absolute alcohol, particularly by removal from filter and shaking in a bottle with the

Precipitation with Pt Cl4.

washing alcohol and re-filtering. It is then drained on bibulous paper, without being permitted to become dry and waxy, or soft and pasty. It is next treated with a large volume of absolute ether until it is differentiated into a fine yellow powder and an orange-coloured solution. These are separated by filtration; the pulverulent matter is washed with ether to exhaustion, which requires its removal from the filter and shaking with ether in a stoppered bottle, and re-filtration, repeated more than once. Ultimately this precipitate dries easily in air, and becomes somewhat waxy, but is easily powdered. It constitutes *myeline-platinic-chloride*. The orange-coloured ether solution is concentrated, when on exposure to cold it will frequently deposit the soluble salt as a solid mass, but it is better to precipitate the solution by absolute alcohol, filter, wash with alcohol, and shake therewith in a bottle two or three times, drain on bibulous paper, and dry in vacuo. The latter precipitate constitutes probably *lecithine-platinic-chloride*. It is, however, less pure than the myeline salt, as it necessarily contains some kephaloidine, from which it has to be purified as will be described under the paragraph to be given to this account.

Treatment of $Pt Cl^4$ precipitate with ether.

Myeline $Pt Cl_4$ from buttery.

Lecithine $Pt Cl^4$ from buttery.

The alcoholic mother liquor from which all matters precipitable by platinic chloride have been removed on standing deposits some cholesterine and a little platinum compound. It is concentrated by distillation, and on cooling deposits much cholesterine. Platinum is now precipitated by hydrothion, and the precipitate extracted with water, which removes hydrochloric acid. From the black precipitate matters are extracted by alcohol and ether, which yield cholesterine and the neutral bodies already referred to; but it is probable that the distillation of the first alcohol in presence of the platinic chloride affects some of the matters, so as to change them more or less from their original properties.

Alcoholic mother liquor.

Yields cholesterine.

Treatment of the last oily.—This is easily soluble in alcohol and ether, without residue. It is simply treated in alcohol solution with platinic chloride, and mostly yields only salt soluble in ether (lecithine ? platinic chloride); sometimes also myeline salt, and sometimes it decomposes during treatment, yielding a resinous platinum salt insoluble in most reagents, and not yet any further studied. It is an accidental product. The mother liquor yields cholesterine and neutral matters, but only in small quantity, being probably oleate and margarate of ethyle. The buttery matter has also been subjected to ether treatment, but without any advantage, and this treatment, which can be easily deduced from the ether treatment of the white matter, as an analogy, need not here be any further described.

Treatment of last oily.

$Pt Cl^4$, from last oily.

Ether treatment, process applied to buttery.

We have thus separated the brain into the following immediate principles or mixtures thereof:—

Principles of brain matter.

1. First extractives (by soaking alcohol).
2. Insoluble albuminous residue.
3. White matter, containing:—
   - *a.* Kephaline (with varieties and compounds).
   - *b.* Myeline (with varieties and compounds).
   - *c.* Lecithine (with varieties and compounds) (?).
   - *d.* Cholesterine (varieties problematical).
   - *e.* Cerebrines (several varieties).
   - *f.* Neutral lipoid matters, probably ethers (several, solid and fluid).
4. Buttery matter, containing:—
   - *a.* Kephaloidine (with varieties and compounds).
   - *b.* Myeline (with varieties and compounds).
   - *c.* Lecithine (with varieties and compounds) (?).
   - *d.* Cholesterine (varieties problematical).
   - *e.* Cerebrines (several varieties).
   - *f.* Neutral lipoid matters, probably ethers (several, solid and fluid).

5. Last oily matter, containing :—
   a. Lecithine (with varieties and compounds) (?).
   b. Cholesterine (varieties problematical).
   c. Etherised lipoid matters (several, solid and fluid)
6. Ultimate watery extract of brain, containing :—
   a. Extractives.
   b. Nitrogenous principles.
   c. Hydrocarbons.
   d. Organic acids and salts.
   e. Inorganic salts.

Explanation of definitions in summary list.

In the foregoing descriptions of processes I have, for the purpose of brevity, spoken of the crude immediate principles which were isolated as if they consisted of a single body each ; but in the summary list just given I have added to most of the names of these compounds an enlarged definition, by which not only *the pure principle*, but also *varieties of it*, and *compounds of it* are said to have been isolated in one and the same operation. This is strictly the case, as will be seen, for example, in the account of kephaline hereafter to be given ; but it may be at once stated that the pure immediate principle which gives rise to the name constitutes the great bulk of each preparation; that this bulk is relatively greatly increased by the removal during the process of purification of matters in chemical combination with a smaller portion of the immediate principle, and that the varieties are very similar in type to the principal matter, and whenever they cannot be separated absolutely their nature and quantity can be ascertained by very good approximations. Where matters are so similar in properties as kephaline and kephaloidine, a complete separation of the entire quantity of each from the other is not easily effected, but a quantity of each can be obtained pure from the other, and from the results obtained by their chemical study the composition of mixtures in any proportions can be derived. The immediate principles above enumerated may be arranged in the following groups :—

Arrangement in groups of immediate principles.

Group of Principles containing Five Elements, C, H, N, S, O.— This may be termed *the group of sulphurised or albuminous principles.* It includes albumen and small quantities of histo-genetic substances.

Group of Principles containing Five Elements, C, H, N, P, O.— This may be termed *the group of phosphorised principles.* It includes:—
   Kephaline, with varieties and compounds.
   Myeline, with varieties and compounds.
   Lecithine, with varieties and compounds (?).

It is at present uncertain whether any of the solid and fluid neutral lipoid matters belong to this group.

Group of Principles containing Four Elements, C, H, N, O.—This may be termed *the group of nitrogenised principles.* It includes :—
   Cerebrine in its several varieties.
   Kerasine in its several varieties.
   Phrenosine in its several varieties.
   Extractive acids, and secretory acids, and their salts, such as uric acid, and congeners.
   Neutral principles, such as urea and amido-acids.

Group of Principles containing Three Elements, C, H, O.—This may be termed the *group of oxygenated principles.* It includes :—
   Cholesterine, with any varieties. } Alcohols.
   Hydrocarbons, such as inosite. }
   Organic acids, such as lactic, formic, &c.
   Fats and fatty acids, which if not regularly found in health, are certainly present in disease.

Group of Inorganic Principles, including both acids and bases, and salts, either free or in combination with many of the foregoing organic principles. This includes :—

Sulphuric acid.
Hydrochloric acid, and as Cl in chlorides.
Phosphoric acid.
Carbonic acid.

Potash, Soda, Ammonia, Lime, Magnesia, Copper, Iron, Manganese — In combination with immediate principles, forming their bases, or in combination with phosphoric acid, and attached to immediate principles as phosphates, or in combination with mineral acids, as free mineral salts in the juices and extracts.

Alumina, silica, fluorine (doubtful).

As the albuminous matters from the brain, when once isolated, offer no specific differences from albuminous matters of the body in general, and as their study, while in combination in the fully constructed brain, can only be carried on with advantage after the matters peculiar to or highly prevalent in brain substance are fully known, I have not given much time to this group, although information as to its bearing in mass, and as to the inorganic matters combined with it in mass, has been obtained; this is to be reserved for expansion in future researches. In the present essay I propose to communicate only those researches which bear upon *the group of phosphorised* and the *group of nitrogenised bodies*, and that *portion of the group of inorganic principles* which directly combines with the bodies of these groups. I may, however, state that much information on the principles constituting all other groups, as well as much material towards their study, has been collected in the course of these researches.

II.—DESCRIPTION OF CERTAIN CHIEF PRINCIPLES WHICH HAVE BEEN ISOLATED.

A.—Group of Phosphorised Principles.

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General properties of the phosphorised principles - - -
Filtration of aqueous solutions - - - - - -
Universal solubility in ether - - - - - -

General Properties of the Phosphorised Principles.—This group comprises kephaline, kephaloidine, myeline, and lecithine, and their congeners and compounds. All these bodies have certain properties in common, which point to some similarity in chemical constitution; by other peculiarities again, they are sharply distinguished from each other. *They are all soluble in water in a certain manner and measure.*

General properties.

When they are in the dry state, and are placed in pure water, they sink to the bottom, and are at once wetted by the water. Thus, their specific gravity is shown to be greater than that of water, and by this and the faculty of being wetted, they are sharply distinguished from the fats or fatty acids, but assimilated to the soaps, as the older authors correctly stated. When they have remained in the water for a short time, they

Manner of dissolving.

begin to swell, to become transparent at the thin margins of the particles, and covered with a loose layer all over their surface. This on agitation is easily detached, and floats in clouds in the liquid; the clouds diffuse themselves indefinitely throughout the whole of the water, and if enough water is present, the mixture is frequently shaken and some time is given, the particles of solid matter disappear entirely and form a

Solution cannot be clarified.

turbid solution of very peculiar appearance. As will be seen hereafter in particular, these solutions can by no mechanical means be clarified, yet they are solutions and pass through many layers of the finest Swedish filtering paper; no particles can be distinguished by high powers of the microscope, yet they must be there and consequently so small as to be beyond the reach of optical definition as single particles. They exhibit their presence, however, by iridescence in the case of myeline and lecithine, and by reflecting polarised light in all cases. These

Similarity to soaps and emulsions.

solutions, therefore, resemble somewhat the cold solutions of soaps, and the emulsions produced by solid fatty acids with neutral phosphates; the emulsion produced by vegetable seeds (almonds) with water, &c.; but they differ from milk and emulsion of fats and gum by there being no particles visible. Moreover, emulsions are supposed to require the presence of two agents besides water, whereas these brain substances give this peculiar solution with mere water; they are not decomposed, as the soaps are, into acid and alkaline salt, as they are not salts, but form these solutions in the free and uncombined, but also in the com-

Definition of imperfect solution.

bined state. I can give no better definition of this peculiar condition than by describing it as *a state of imperfect or incomplete solution*, a stage intermediate between the solid and the fluid state of matter. Those who do not coincide in this description may term the solution an "emulsion," if that conveys any definite idea, or a state of the finest subdivision of particles with peculiar attraction of these particles to water, and consequent repulsion of particles from each other. This latter part of the question I shall have to consider at greater length when I come to discuss the dependence of the structure of the brain upon the chemical and physical characters of its ingredients. For the present purpose it suffices to sum up that these phosphorised bodies have all an extreme attraction for water, swell in it, and ultimately form a nearly perfect solution. By this means they afford good opportunities for mechanical and chemical purification now to be described.

Deposition of impurities.

Deposition of Impurities.—The solutions on standing deposit any mechanical impurities, and any cholesterine contained in the matters dissolving crystallises out and sinks to the bottom. Any excess of the matters beyond saturation is also deposited, as well as the less soluble compounds. If any cerebrine were with the matters it would deposit as a thick gelatinous matter. All solutions therefore produced as above, and which experience has shown me, should not contain above one per cent. of the matters, must be allowed to stand for a day or two, in order that these insoluble impurities and admixtures may be deposited. The

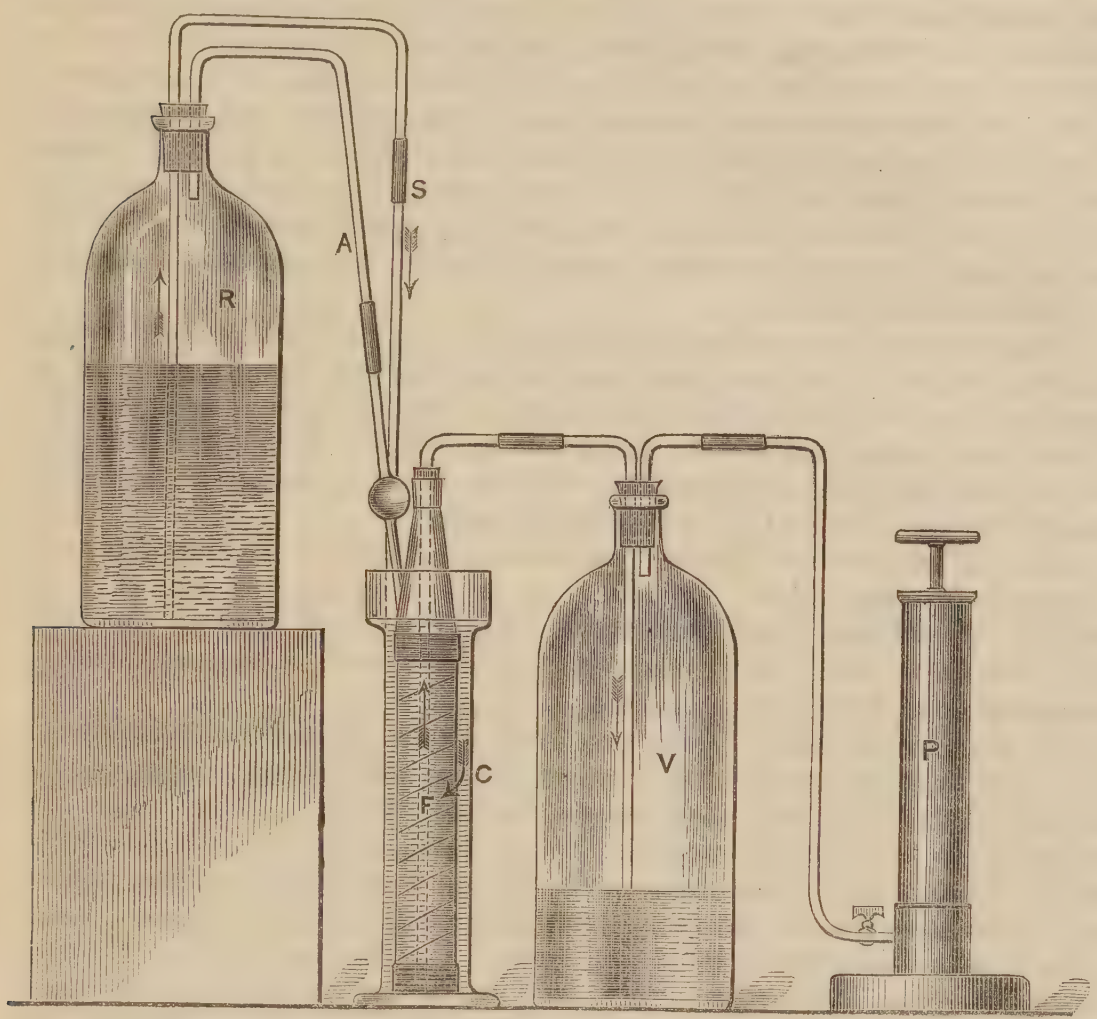
Decanting by syphon.

pure solution is then removed with the syphon from the deposit; the solution is filtered, and the deposit further exhausted with water.

Filtration of aqueous solutions.

Filtration of the Watery Solutions of the Phosphorised Matters.—When the solutions are placed on an ordinary filter of paper, a portion passes by gravitation, but the pores of the paper gradually become ob-

structed and filtration ceases. It is therefore necessary to expedite filtration by the aid of pressure. For this purpose I have constructed a new apparatus in which a vacuum draws the liquid to be filtered into a hollow cylinder surrounded with paper.



Apparatus for Filtration by Air-pressure.

The filter F is a hollow cylinder of silver-plate pierced like a fine sieve, and covered with a six-fold roll of Swedish filtering paper, made secure at the top and bottom by a ring of string, which also runs spirally over the whole paper. This stands in a glass cylinder C which contains the fluid to be filtered and is always kept full by a perpetual siphon S, drawing the liquid from the reserve bottle R ; as frequently as the fluid in the wide upper part of the cylinder sinks below the oblique end of the air tube A, which is wide and provided with a bulb, air is admitted by A into R and the siphon acts. The bottle receiving the filtrate V is evacuated of air gradually by means of the air pump P. The tube which draws the filtered liquid from the cavity of the cylinder goes to the bottom of the cylinder, so that nearly the whole of the filtered liquid can at any time be drawn into V.

Such a cylinder, about a foot long and four inches in circumference, will, with a pressure on C of 70 centimetres of mercury, filter a Winchester quart full of one per cent. solution of phosphorised matter per day ; the more, the purer the substance is, the less, the more cholesterine and cerebrine it contains. Sometimes only 100 cc. are filtered per hour. In such a case a coarser filtering paper should be rolled over the Swedish to collect the coarsest particles and prevent them from getting upon the Swedish paper. After some hours filtration becomes slow and ultimately ceases entirely ; then the paper is found covered with a gelatinous mass of undissolved matter and impurities. These may be rinsed off and

Quantity filtered in time.

again extracted and filtered; but I have mostly found the matter so small in quantity that I have discharged it with the paper. Such a filter, with two changes of paper per day, may be going night and day, and if all corks are air tight, which is easily effected by applying hot paraffine to them, requires very little attention except a few strokes of the pump from time to time. When the substances previous to their solution in water were very pure, they left no vestige of cholesterine crystals, or of cerebrine on the filter. The filtered fluids are still opaque, those from kephaline more colored, those from myeline and lecithine of a milky whiteness with the blue iridescence. When a good filtration had been effected, a second filtration effected no improvement and was quickly accomplished. In this manner all phosphorised matters used for cardinal preparations were passed through the process of watery solution and filtration.

Filtered fluids.

The process offers peculiar advantages for each of the three varieties of phosphorised principles, but is essential only for the purification of myeline from cerebrine; this separation is effected more conveniently, if not more completely, than by means of ether. Kephaline scarcely ever retains any cholesterine, but myeline always does, unless it has passed through the $Pt\,Cl_4$, or $Cd\,Cl_2$ process.

Solubility in ether.

All the Phosphorised Matters are soluble in Ether, but in very different Proportions.—Kephaline dissolves in this reagent in almost any quantity; lecithine only less than kephaline; but myeline is very little soluble in cold ether, more in hot ether, and is instantaneously deposited from the ether as it cools.

Solubility in alcohol.

All the Phosphorised Matters are soluble in Alcohol.—Lecithine in all proportions in hot absolute alcohol, less in cold; kephaline is almost insoluble in cold alcohol, more soluble in hot, almost entirely deposited on cooling; myeline, however, is little soluble in cold alcohol, largely soluble in hot, and is deposited on cooling in a crystallised state, and in such quantities that the fluid becomes filled with crystals. It will thus be seen that while water offers no means for the separation of these substances from each other, ether and alcohol offer great advantages, which have indeed been utilised in the construction of the method for their separation above described.

(*a.*) *Sub-Group of the Kephalines.*

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Purification by resolution.

Kephaline.—$C_{42}\,H_{79}\,N\,P\,O_{13}$.

Purification.—The crude kephaline obtained by the primary operations is dried under the air pump over sulphuric acid. It must be

repeatedly taken out, and flattened out in a mortar, and again dried, before it becomes dry and brittle and can be powdered. Resolution in absolute ether, reprecipitation by absolute alcohol, and redrying causes a great improvement, but also much loss.

Filtration of the solution in water.

The dry substance is then dissolved in pure water, 10 grms. in the litre, and after complete solution by agitation in a stoppered bottle, is allowed to deposit less soluble salts and impurities, decanted or syphoned from these, and then filtered by pressure as described.

Treatment with HCl.

The filtered solution is now treated with enough hydrochloric acid to effect complete precipitation of all kephaline as hydrochlorate, a salt which appears in voluminous flakes, and on shaking collects on the surface of the liquid. (This precipitation, like others to be related, affords a good criterion of the previously dissolved state of the substance.) The mother liquor is drawn from underneath the precipitate with a siphon; fresh water is poured on the precipitate, and the whole is now placed upon a paper filter or cloth, and washed with water, until it begins to swell and dissolve. At that point it is found that all HCl is washed out, and that only *pure hydrated kephaline* remains on the filter. This was expressly proved by analysis in several cases, but more especially in the following experiment.

Washing with water.

Experiment proving Expulsion of Hydrochloric Acid from Kephaline by Water.

Experiment proving expulsion of H Cl from kephaline by water.

Half a litre of a weak kephaline solution was precipitated by H Cl and filtered; the precipitate on filter was replaced in 500cc. of water to test its solubility, and was found insoluble, but the water contained free H Cl. It was again filtered, and after solution had ran through, absolute alcohol was blown on the precipitate; this became adhesive, and the solution refused to filter. The alcohol and precipitate were transferred to a beaker, and the whole was gently warmed in a water bath to a temperature not much above 60°. The precipitate fused, but dissolved very sparingly, apparently the less the more the alcohol made the precipitate anhydrous. The alcoholic solution was now filtered off warm, and the undissolved portion of the precipitate taken up with ether. The solution so obtained was of a colour resembling ordinary kephaline in ether, and was poured into the alcoholic solution. The precipitate which ensued was fawn coloured, adhesive, and leathery; it was isolated, washed with absolute alcohol, the mother liquor being squeezed out of it, then pressed and dried in air-pump. When dry it weighed 0·62ºº gram. The whole was analysed for chlorine as follows. It was heated to dryness in a strong solution of pure soda, with carbonate and nitre added, and the mass was burned. The fused mass was dissolved in dilute nitric acid, and the solution tested with argentic nitrate. No precipitate occurred, showing that *the body obtained in this process is not a hydrochlorate*, but probably kephaline in a purified state. This latter probability is raised to a certainty by the following experiments.

Traces of ammonia.

Examination of the Bases and Salts which are in combination with Kephaline, after Filtration of its Aqueous Solution. — The solution of hydrochloric acid and other matters filtered from the precipitated kephaline was evaporated to dryness. A portion was then boiled with solution of baryta, when traces of ammonia were evolved. The rest of the residue was then ignited in a platinum dish to destroy all traces of organic matter. The ash was slightly molten, and only partially soluble in water, but easily soluble in water slightly acidified with hydrochloric acid. The solution was filtered from a trace of

carbon, and treated with excess of ammonia, whereupon an abundant precipitate of *earthy salts* fell down, and the solution assumed a *deep blue colour*. Precipitate and solution were separated by filtration. The precipitate dissolved readily in a little HCl, forming a slightly red solution, indicating presence of *iron*, which was confirmed by the sulphocyanide test. In another portion of the solution dilute sulphuric acid gave an abundant precipitate of gypsum, showing presence of *calcium*. In another portion the molybdate test showed the presence of *phosphoric acid*. To the remaining portion a few drops of ferric chloride were added, then sodic carbonate nearly to neutrality, and lastly, excess of barytic carbonate. This mixture was allowed to stand, filtered and filtrate, freed from excess of baryta by sulphuric acid, filtered and filtrate after supersaturation with ammonia, gave a great precipitate with ammonium oxalate, insoluble in acetic acid, showing presence of much calcium. The filtrate from this calcic oxalate on concentration and treatment with ammonia, ammonic chloride, and sodic phosphate gave the precipitate characteristic of *magnesium*. The precipitate produced above by barytic carbonate was boiled with excess of pure soda, and filtrate warmed with ammonic chloride, when only a turbidity was produced, indicating the *absence* of *aluminium*.

The precipitate contains iron.

Calcium. Phosphoric acid.

Calcium confirmed.

Magnesium.

No aluminium.

The alkaline filtrate from the foregoing precipitate by ammonia was tested for lime by oxalate, when a considerable precipitate was produced, showing the *presence of lime uncombined with phosphoric acid*, and which must therefore have been in combination with part of the kephaline. The blue solution was again filtered from calcic oxalate, which had been entirely precipitated, and acidified with hydrochloric acid. The *copper* was precipitated by hydrothion, the filtrate evaporated to dryness, ignited, and the residue tested for alkalies. This residue was considerable, surmounting in quantity or bulk the bases previously removed. It was fusible with ease, and on solidification became white and crystalline, but interspersed with many red particles of ferric oxyde. The fusion showed it to be mainly *potassic chloride*, but there was also some *sodic chloride* present, as indicated by flame reaction, and the ferric oxyde which had escaped precipitation by excess of ammonia.

Lime not in combination with P_2O_5.

Copper.

Potassium sodium.

Summary.

It is therefore proved that the kephaline obtained by the alcohol and ether processes, and purification by solution in water and filtration, consists of the following ingredients:

Kephaline in the free state.

Kephaline combined with ammonium, sodium, potassium, calcium, iron, copper, and with calcic and magnesic phosphates.

This experience was repeated a great number of times on, in the aggregate, several hundred grammes of dry kephaline; the calcic and potassic salts were always found prevailing greatly in quantity over the others, none were ever absent. Although I have not compared the total of the neutralising power of the bases with the total of the acid combining power of the kephaline, by direct quantitative experiment, I am sure that much kephaline must have been present in the free state, as will appear from future developments concerning the combining and dissociating powers of this body.

Attempted clarification and decolorisation of kephaline.

Experiments intended to effect a Clarification and Decolorisation of Watery and Ethereal Solutions of Kephaline.

White of egg does not curdle in kephaline solutions.

To a solution of one gram kephaline in 100 c.c. of water, 5 c.c. of filtered fresh *white of egg* were added, and the mixture was heated in a flask in the water bath; it remained turbid and no separation of coagulated

albumen took place. The mixture had a faintly alkaline reaction. On addition of a drop of acetic acid to the heated mixture a copious precipitate ensued, which enclosed both kephaline and albumen (the kephaline solution by itself, cold, is only partially or imperfectly precipitated by the same acetic acid). By filtration a perfectly clear liquid was obtained, which was no longer precipitated by baryta water or platinic chloride, and not changed by boiling. Consequently, all albumen and all kephaline were removed together from the solution and perhaps in part combined. Cold absolute alcohol in large quantity extracted all or nearly all kephaline from the albumen, and on distillation left it *perfectly white*. The albumen, on the other hand, was, after washing with alcohol and ether, in a finely divided pulverulent state, and not hard nor horny. This process is therefore useful for preparing snow-white kephaline, which must however not again be brought into contact with ether, as that would immediately cause it to become coloured under the influence of oxydation.

Acetic acid curdles. mixture. Kephalate of albumen.

White kephaline.

Porous albumen.

This experience bears directly upon the treatment of albuminous solutions from animal liquids ; for it must now be studied whether they do not all contain kephaline and allied bodies, and whether the phosphorus so often found in them is not due to the admixture of a quantity of kephaline.

Question whether all animal albumens contain kephaline.

Influence of Animal Charcoal on Water Solution of Kephaline.— To a solution of 1 grm. kephaline in 100 c.c. of water, 2 grms. of pure animal charcoal were added, the mixture shaken, and then subjected to the vacuum filter. A little fluid passed, which became at last quite clear. A portion of the last clearest, collected by itself and tested, was found to be almost pure water ; for hydrochloric acid, platinic chloride, baryta hydrate, and lead acetate, produced the very feeblest precipitates only, while the original solution was made solid by the same precipitants. The animal charcoal therefore retained the kephaline, and when isolated and extracted with alcohol yielded it up to that solvent. This experience, as well as that made with albumen, shows that the watery solution of kephaline cannot be clarified by these agents, if indeed they do not show also that the condition of kephaline is one of suspension and not of true solution. That, however, charcoal has a special attraction for kephaline, such as it also exhibits towards other ammonium bases and alkaloids of undoubted solubility in water, is shown by the following experiment.

Animal charcoal retains kephaline.

Yields it to alcohol.

Bearing of Kephaline in Ether with Charcoal. — A concentrated solution of kephaline in ether was treated with much animal charcoal, in order to be decolorised. The object was but partially obtained. The charcoal, after filtration and washing, was found to retain much kephaline, which was extracted by boiling absolute alcohol, and from this deposited on cooling in a *perfectly white state*. The solution deposited more on spontaneous evaporation. Both were tested and identified as kephaline.

Charcoal extracts kephaline from ether.

Yields it to alcohol.

Kephaline therefore can be removed from watery solution by charcoal and curdling albumen, and again extracted from these substances by hot or cold alcohol in large quantity, and obtained from these solutions in a perfectly white state.

Summary

Ultimate Analysis of Kephaline.—A specimen of highly purified kephaline was passed through the water-filtration and hydrochloric acid process ; it amounted to four litres of one per cent. solution, and after re-solution in ether and precipitation by alcohol left about 30 grms. dry matter. It was thoroughly dried in vacuo over sulphuric acid, being frequently triturated, and ultimately reduced to a fine powder. Carbon

Elementary analysis.

C and H determination.

and hydrogen were determined by combustion with lead chromate and copper turnings. 0·4622 grm. gave 1·017 grm. CO_2, equal to 0·2772 grm. carbon or 60·00 °/₀ carbon ; and 0·391 grm. H_2O, equal to 9·399 °/₀ hydrogen.

Nitrogen.

Nitrogen was determined by volume, the bichromate and carbonate mixture being used for production of carbonic acid gas. 0·544 grm. gave 8·0 c.c. gas at 15° C and 763 m.m. B. Height of K H O column =150 m.m. equal to 15·33 m.m. mercury. Total 28 m.m. deducted from 763 leaves 735 m.m. $\frac{8 \times 273 \times 735}{288 \times 760} = 7{\cdot}33$ c.c. normal $= 1{\cdot}68$ °/₀ nitrogen.

Phosphorus.

Phosphorus was determined by evaporating the substance with solution of pure soda, made from metallic sodium, mixed with carbonate and nitre, to dryness, slowly deflagrating, &c., and determining phosphoric acid by magnesia method. 0·9574 grm. gave 0·1468 grm. magnesium pyrophosphate ($Mg_2P_2O_7$), equal to 4·27 °/₀ phosphorus.

Thus we have the following per-centages and atoms :—

	Found.	÷ by at. wgt.	÷ by N = 1	Theory. Atoms.		Theory. Per cents.
C	60·00	5·00	41·7	42 C	504	60·28
H	9·39	9·39	78·3	79 H	79	9·44
N	1·68	·12	1·0	N	14	1·67
P	4·27	·13	1·0	P	31	3·70
O	24·66	1·54	12·9	13 O	208	24·88
	100·00					

Formula.

leading to formula $C_{42}H_{79}NPO_{13}$.

We shall see hereafter that this formula is supported by the results of the analysis of a number of other preparations, being partial or complete compounds of kephaline, of which the organic matter always has the composition of the free substance ; and by the results of a series of chemolytic experiments the sums of the cleavage products of which also lead to this formula.

Combines with water.

Combinations of Kephaline.—In water kephaline swells and forms an emulsion, ultimately an imperfect turbid solution. Its affinity for water is very great, and the last quantity of water is expelled from it in the vacuum only with great difficulty and after a long time. From a watery solution or mixture it cannot be extracted by ether, as the liquids form an emulsion which persists even after a portion of the ether has separated from the water. This emulsion is very thick, like paste, white like milky water, and practically unmanageable. When a drop or a few drops of a concentrated ether solution are allowed to fall into a test tube full of water, the mixture is at once transformed into a white jelly, which is so firm that the tube can be turned upside down without anything flowing out of it. Solutions of kephaline in water on standing do not decompose or become mouldy, even in the course of some weeks.

Ether makes a jelly ; no extract obtained.

Solubility in alcohol.

Cold absolute alcohol dissolves a little kephaline, more on boiling, and deposits a part on cooling in white flocks. 100 parts absolute alcohol at 17° C. dissolve seven parts kephaline ; at boiling heat of the alcohol nine parts, of which two parts are deposited on cooling. When an excess of kephaline is boiled with an insufficient amount of alcohol,

the part which remains insoluble does not seem to undergo any change, for it retains its solubility in ether and precipitability by alcohol and other reactions.

In ether kephaline, when not too much hydrated, is highly soluble ; when dry it is soluble in anhydrous ether in almost any proportions ; it does not crystallise from this solution, and cannot be made to deposit as from a mother liquor. It is precipitated from the ether solution by an equal or greater volume of alcohol in white clouds, which combine to clots, and ultimately forms a firm substance, which becomes at first plastic, and then dries in vacuo to a hard brittle mass. The ether solution becomes quickly red in transmitted light, and fluoresces with a fine green color. No other phosphorised or other brain ingredient shows this peculiarity except kephaloidine.

Solubility in ether.

Alcohol precipitates ether solution.

Fluorescence.

Solubility of Kephaline in Benzole.—A specimen, thrice precipitated from ether by alcohol, and when last in ether, exposed during 24 hours in ice to a temperature of 0°, proved soluble in cold benzole, and very soluble in hot. It formed a yellow solution. A sample in a test-tube exposed to frost gave no deposit. The addition of alcohol to the benzole solution produced a slight precipitate, insoluble in excess of alcohol, but soluble in excess of benzole, and soluble on heating. Benzole can therefore not be used, like ether, for the purification of kephaline.

Solubility in benzole.

Precipitated by alcohol from benzole.

Reactions of the Aqueous Solution of Kephaline.—A one per cent. solution, filtered by air-pressure through three-fold Swedish filter paper, was used.

Reactions in test tubes.

1. Hydrochloric acid gives a bulky curdy precipitate, slightly yellow, and after isolation soluble in ether, *not* precipitated by alcohol from its ethereal solution. In the filtrate from this hydrochloric acid precipitate platinic chloride produces the merest opacity. But from the ethereal solution of the H Cl precipitate alcoholic $Pt Cl_4$ throws down a precipitate, which is soluble in ether, and reprecipated by alcohol. — H Cl.
2. Sulphuric acid produces a precipitate like that produced by H Cl. — $H_2 S O_4$.
3. Nitric acid the same as the previous acids. — $H N O_3$
4. Baryta water produces a bulky curdy precipitate. — $Ba H_2 O_2$.
5. Lime water produces a similar precipitate, but it does not separate like the $Ba H_2 O_2$ precipitate. — $Ca H_2 O_2$.
6. Cadmic chloride, curdy precipitate which readily coalesces. — $Cd Cl_2$.
7. Zinc chloride, similar to $Cd Cl_2$. — $Zn Cl_2$.
8. Mercuric nitrate produces a dense precipitate, which is insoluble in nitric acid, but colored slightly yellow thereby, heat being evolved. The precipitate is sometimes rose red, and in adhesive flakes. Washed and allowed to stand with water it becomes again white, ropy, and adhesive, and soft, and on being shaken easily dissolves in water in the manner of the original kephaline. The mercuric nitrate seems therefore to be separated by water from kephaline in the same manner as other salts and acids are. — $Hg N O_3$.
9. Baryum chloride produces a good dense flaky precipitate. Immediately after isolation it is insoluble in water, insoluble in alcohol, but easily soluble in ether, and apparently reprecipitated by alcohol. This reprecipitated matter was proved to contain baryum. Some special experiments with this compound will be related hereafter. — $Ba Cl_2$.
10. Calcium chloride acts like $Ba Cl_2$. — $Ca Cl_2$.
11. Platinic chloride produces a bulky precipitate. — $Pt Cl_4$.
12. Ammonia makes solution a little turbid ; no precipitate. — $N H_4 H O$.
13. Platinic chloride mixed with H Cl produces a very well-defined precipitate. — $Pt Cl_4 + H Cl$.

14. Magnesium chloride, a very precise immediate precipitate. — $Mg Cl_2$.
15. Ferric chloride, a yellowish turbidity ; imperfect precipitate. — $Fe_2 Cl_6$,
16. Uranic nitrate, a white turbidity, and precipitate imperfect. — $Ur N O_3$.
17. Watery bromine produces a bulky precipitate, nearly whit soluble in caustic potash ; acetic acid added to this agai, liberates the precipitate. Chloroform added to this mixture produces a chloroform solution of Br at the bottom, containing the excess of reagent, and an impracticable white emulsion on the top. — Br.
18. Cupric nitrate — $Cu (N O_3)_2$.
19. Cupric chloride — $Cu Cl_2$.
20. Cupric sulphate — $Cu S O_4$.
21. Cupric acetate — $Cu (C_2 H_3 O_2)_2$.

All produce perfect precipitates of a greenish white colour.

22. Mercuric chloride makes the solution very turbid, but no precipitate. — $Hg Cl_2$.
23. Mercuric acetate, immediate complete precipitate. — $Hg (C_2 H_3 O^2)_2$.
24. Argentic nitrate, immediate complete precipitate, darkening a little when exposed to sunlight. — $Ag N O_3$.
25. Auric chloride, and a drop of H Cl, immediate precipitate, which blackens over night. — $Au Cl^3$.
26. Antimonic chloride, a very bulky precise white precipitate. — $Sb_2 Cl_3$.
27. Stannous chloride, a white flaky complete precipitate. — $Sn Cl_2$.
28. Stannic chloride, a precipitate and turbid solution. — $Sn Cl_4$.
29. Tannin in water, no particular reaction. — Tannin.
30. Picric acid, a turbidity, but no manageable precipitate. — Picric acid.
31. Arsenious acid, a precipitate and turbidity. — $As_2 O_3$.
32. Arsenic acid, a very complete immediate precipitate. — $As_2 O_5$.
33. Phosphoric acid, a very complete immediate precipitate. — $H_3 P O_4$.
34. Basic lead acetate, a precipitate and very turbid solution; no perfect separation. — Basic Pb acetate.

In none of the foregoing reactions was any artificial heat employed, but they were all made at the ordinary temperature.

It was found that most of these precipitates could not be washed with water without losing either acid or base, or salt, with which they were combined. But most of them remained insoluble in water until the point of purity was reached, when the kephaline either dissolved in the pure water, or clogged the filtering paper.

Dialysis of kephaline.

Dialysis of Kephaline.—A solution of five grams of crude kephaline was dissolved in 500 c. c. of water, and formed a white thick liquid, from which some cholesterine crystallised on standing. It was then placed on a dialyser of parchment paper. A trace of kephaline passed into the water, but so small was the quantity, that no chemical operation could be undertaken with it.

Kephaline does, practically, not dialyse, but may perhaps act as a colloid, and allow its impurities to pass out into the water.

Kephaline cadmic chloride salt

Kephaline Cadmic Chloride Salt, prepared from Kephaline purified from bases by H Cl Process.—Two litres of a filtered one per cent. solution were precipitated by dilute H Cl. The mother liquor was drawn off, and the precipitate washed by agitation with water. The washing water was again drawn off, and watery solution of cadmic chloride added, which caused great condensation of the precipitate. The liquor was again drawn off, and the precipitate shaken violently with a great quantity of alcohol containing alcoholic cadmic chloride. Thus the precipitate was condensed to a viscous mass, from which the mother liquor was drawn off, and the alcohol entirely removed by careful manipulation. After draining, the precipitate was dissolved in ether,

and to the ethereal solution was added alcoholic cadmic chloride, cautiously, till a slight permanent precipitate was perceived. This was removed by filtration, and the brilliant fluorescent filtrate precipitated by absolute alcohol. The viscous mass was again drained from all alcohol by pressure with a glass rod. Redissolved in ether, it formed a perfectly clear solution, which was reprecipitated by absolute alcohol, when the compound came down in an almost pulverulent state. It was thrown on a filter, washed with absolute alcohol, then removed on a glass dish and placed under a dryer, then in a vacuum over sulphuric acid, and frequently removed to be powdered in a mortar, and ultimately finely pulverised. It was then subjected to elementary analysis, which yielded the following results:

Elementary Analysis.

1. Carbon and hydrogen were determined by combustion with Pb Cr O_4, and copper turnings. 0·4630 gram. gave 0·9100 C O_2, equal to 53·603 °/₀ carbon, and 0·355 H_2O, equal to 8·519 °/₀ hydrogen.

   Carbon and hydrogen.

2. Nitrogen determined as gas. 0·5504 grm. gave 6.5 c.c. N, at 14° C and 765 m.m. B. Height of K H O column = 146 m.m.

   Nitrogen.

   These elements give the calculation $\frac{6\cdot5\times273\times739}{287\times760}=6\cdot01$ c.c.N normal = to 1·37°/₀ nitrogen.

3. Chlorine, cadmium, and phosphorus were determined by fusion with caustic soda, nitre, and carbonate, with great caution; solution of salts in acid, precipitation of cadmium by hydrothion, and conversion into carbonate. Chlorine and phosphorus were determined in filtrate from cadmic sulphide by the usual methods. 1·0022 grm. salt gave 0·0742 Cd O, equal to 6·47 °/₀ cadmium. Further, 0·1682 grm. Ag Cl, equal to 4·15 °/₀ chlorine, and 0·1268 grm. $Mg_2P_2O_7$, equal to 3·54 °/₀ phosphorus. The cadmium and chlorine were in the relation of Cd : Cl_2, inasmuch as 4·15 parts chlorine require 6·54 parts cadmium.

   Chlorine, cadmium, and phosphorus.

Summary of results—

Summary.

C	53·603		
H	8·519		
N	1·37	89·38	
P	3·54		
O	22·35		
Cd	6·47		
Cl	4·15	10·62	
	100·00	100·00	

Calculation shows that the kephaline is not completely saturated with Cd Cl_2, ; that about four parts out of nine are uncombined. For the formula, derived from the organic matter with P as 1, *i.e.*, $C_{42}H_{79}NPO_{13}$, Cd Cl_2 yields the equation:—

$$\underbrace{C_{42}H_{79}NPO_{13}, CdCl_2}_{1019} : \underbrace{CdCl_2}_{183} :: 100 : 17\ 95,$$

whereas only 10·62 °/₀ Cd Cl_2 were found.

Deducting $CdCl_2$, and calculating per cents. of elements in organic body, we get—

		÷ by At. Wgts.	÷ by P as 1.	At. Wgts.
C	59·97	4·99	41·6	504
H	9·53	9·53	79·4	79
N	1·53	·11	1·0	14
P	3·96	·12	1·0	31
O	25·00	1·56	13·0	208
				836

leading to formula $C_{42}H_{79}NPO_{13}$, with an atomic weight of 836, thus fully sustaining the composition of the free substance.

As 10 parts of $CdCl_2$, supposed to be combined with a molecule of kephaline correspond to 59 parts of compound, about 40 per cent. of the above substance may have been free kephaline.

The very weak chemical affinities of kephaline are here exhibited in a striking manner; $CdCl_2$ was brought into contact with it at various periods, and yet could not be retained in combination. This is due to the fact that $CdCl_2$ cannot be dissolved in absolute, but only in somewhat watery alcohol; and even little water decomposes the compound, and carries the $CdCl_2$ away, as will be shown by special experiment hereafter.

First preparation.

Bearing of Kephaline with Hydrochloric Acid and Platinic Chloride.—About three litres of 1 °/₀ solution, which by the deposition of the insoluble part and filtration had lost much of the 1 °/₀ originally dissolved, were treated with a mixture of HCl and $PtCl_4$ in slight excess. A bulky yellowish white curdy precipitate ensued, and rose to the surface; it was allowed to contract, and the yellow mother liquor drawn from beneath it by a syphon. Absolute alcohol, equal in bulk to the precipitate, was now poured upon it, and the mixture violently shaken. This caused a further contraction of the precipitate, indicated by lesser bulk, increase of the yellow colour, and production of adhesiveness. The alcoholic liquid was again syphoned off, and another quantity of absolute alcohol was now poured on the precipitate, and the mixture violently shaken. The precipitate thereby became deep yellow, adhering to the glass, and so contracted that the liquor could be poured off quite clear. The precipitate was now treated with a minimum of ether, in which it proved quickly and entirely soluble. On filtration, nothing whatever remained on the filter. An equal

Solution in ether.

volume of absolute alcohol was added to the ether solution, whereby almost the whole of the salt was precipitated. The latter was freed from mother liquor by careful manipulation with a glass rod, redissolved in absolute ether, which gave a brilliant solution requiring no filtration, and this was reprecipitated by absolute alcohol in equal volume, added in a thin stream while the liquid was being stirred. The mother liquor was poured off; the precipitate, which immediately became brittle and hard on rinsing with absolute alcohol, was drained from alcohol and placed over sulphuric acid in the vacuum.

Second preparation.

Second Preparation.—Another amount of the same body was made from four litres of a 1 °/₀ solution, in the same way, with this difference, that whereas in the first case the precipitate was washed with alcohol directly, in *this* case two washing with water were carried out before the application of alcohol. This was done in order to test the effect of water upon the compound desired to be produced. It was found that the dry body treated with ether and alcohol like the first

preparation contained only a trace of platinum and a vestige of chlorine, both elements being too small in quantity for accurate determination.

Both preparations were therefore united, and it was sought to combine them with platinic chloride under circumstances, where the influence of water was as much as possible excluded. (It must be remembered that solid platinic chloride contains six atoms of hydration water, which it necessarily carries into all its solutions.) They were dissolved in ether and an ethereal solution of $Pt\,Cl_4$ was added, then precipitated with absolute alcohol. Again dissolved in ether, and treatment with $Pt\,Cl_4$ repeated. Ultimately the precipitate was dissolved in pure ether, reprecipitated by pure alcohol, and dried in vacuo.

Contains only a trace of Pt Cl4. Preparations united.

Analyses.—1·581 grm. deflagrated with the usual precautions yielded 0·0568 grm. metallic platinum, equal to 3·592 °/₀ Pt, and 0·2088 grm. Ag Cl, equal to 3·265 °/₀ Cl.

Analyses.

If the platinum had been accompanied by only as much chlorine as corresponds to tetrachloride, 2·58 °/₀ Cl should have been found. By calculation we find that there is one fifth of Cl more than corresponds to this proportion, viz., 1 Pt : 5 Cl=3·592 Pt : 3·22 Cl.

A compound of the presumable formula :

$2\,(C_{42}\,H_{79}\,N\,P\,O_{13}) + 2\,Cl + Pt\,Cl_4$ with an atomic weight of 2083 requires 9·5 °/₀ Pt and 10·2 Cl. Consequently, the platinic chloride compound comprises about one third of the kephaline, of which two-thirds are uncombined.

It is thus seen that although kephaline is most completely precipitated by $Pt\,Cl_4$ from its watery solution, yet by the process of solution and precipitation with solvents in which $Pt\,Cl_4$ is soluble, most of the $Pt\,Cl_4$ is extracted from the combination, and lost in the mother liquors, just as it is almost entirely extracted from the same precipitate by water. In short, the acids, bases, and metallic salts which easily combine with kephaline, when they are present in excess, are rapidly separated from it by solvents in which they themselves are readily soluble.

Summary.

Chemically speaking these results are disappointingly negative, inasmuch as they refuse to furnish the ordinary means for determining the atomic weight and for finding guarantees of purity of preparations. But physiologically these features are of the greatest interest, inasmuch as they show us a marvellous diversity of power of reaction of kephaline, by entering into and out of combination according to external circumstances. When the combinants are offered in a concentrated state they are retained, when the liquids which carry the combinants (blood, serum, cerebrospinal fluid) become again diluted, the combined matters must again pass into solution and travel further. Thus every change of chemical composition of the juices of the body must necessarily and powerfully affect the condition of the brain and nerves, and of all tissues and cells containing their specific ingredients.

Theoretical considerations.

Condition of labile equilibrium.

Kephaline and Baryum Chloride.—Two litres of a 1 °/₀ watery filtered solution of kephaline were precipitated by $Ba\,Cl_2$, the mother liquor was drawn off, the precipitate agitated with water containing $Ba\,Cl_2$, then washed with pure water, and finally on a filter where the washing with water was repeated until the washings were free from baryum and chlorine. The enormously hydrated and swelled frothy precipitate was placed under a drier over sulphuric acid and dried, being frequently stirred and moved, ultimately powdered in a mortar to change the drying surface of the hardening matter and the drying completed in vacuo.

Kephaline and Ba Cl2.

Analyses.

Analysis showed that the kephaline was almost pure, and retained only traces of baric chloride.

Heating to 100° C.

Decompositions of Kephaline.—When pure kephaline in the perfectly dry state is heated in a water oven to between 90° and 100° it fuses into a dark red transparent viscid oil. It becomes solid again on cooling, but retains a viscosity, so as to adhere to the fingers, which it did not before it was heated. Treated with water the heated and cooled kephaline swells again and gradually dissolves, but is darker coloured, so that some slight degree of decomposition or of oxydation seems to have taken place.

Ignition.

When heated to higher temperatures it gives out much heavy strongly smelling inflammable vapour, which partially burns with formation of much soot. Ultimately a bulky charcoal is left, which cannot by ordinary heating in platinum vessels be entirely destroyed, as it is soaked with phosphoric acid. It cannot be entirely burned even after the principal quantity of phosphoric anhydride has been extracted with water. Complete combustion is effected only in the presence of nitre.

H_2SO_4+sugar test.

With concentrated sulphuric acid dry kephaline in fine particles immediately assumes a dark red brown colour, which gradually becomes nearly black. When sugar and sulphuric acid are allowed to act upon kephaline a reaction similar to the one given by bile acids is gradually engendered. But the process essentially requires time, during which the mixture passes through a stage of dark brown colour, until a deep purple is at last attained. But this colour is never so pure or beautiful as that obtained with cerebrine, myeline, or the bile acids. It is therefore probable that a decomposition of the kephaline has to be effected before the reaction is attained.

Chemolysis by Na H O

Chemolysis of Kephaline by Caustic Soda. First Experiment.—A watery solution containing 30 grms. of pure kephaline, filtered, &c., was precipitated by H Cl; the mother liquor was syphoned off, and the residue washed once with distilled water. The H Cl necessarily yet contained in the precipitate was disregarded, as not interfering with future operations. The pulpy deposit was put into a flask, and a solution of 5 grams. crystallised soda hydrate added. The precipitate disappeared on agitation, forming first a gelatinous, later a fluid solution, similar to the filtered kephaline solution. The solution was now placed in a bath and heated gently for nine hours. During the process it was observed that skins formed on the surface similar to membranes on milk while being heated (? May such a phenomenon have given rise to the surmise that the yolk of egg contained "caseine"?) On cooling the liquid formed a gelatinous cake, consisting of viscous curds set in a thinner fluid. Next day the mixture was boiled during nine hours on a sand bath. Bumping was mitigated by dropping a spiral of platinum into the fluid. Great frothing ensued, which was opposed by a funnel fixed upon the top of the flask by a cork. The funnel was supported by a stand to prevent its breaking the flask in the event of itself breaking. (Eventually both funnel and flask cracked, the latter at the base of the neck.) After this boiling the liquid was still turbid, became gelatinous, viscid, and set on cooling, and covered by a membrane, and was supposed to be decomposed. On commotion it showed the wavy glistening appearance of soaps, and when at repose in a beaker formed folds as from a tubular membrane sinking.

Reactions of soapy solution.

Reactions of the Soapy Solution and Mixture.—It was insoluble in water, and not further precipitated thereby. It was curdled by cold alcohol, dissolved by hot, leaving, however, some particles undissolved, which proved soluble in ether.

Not precipitated by concentrated sodium chloride solution, *i.e.*, not salted out. Acetic acid added to this last sodium mixture causes curdling, and on boiling the curds collect on the top without fusing. Consequently, none of the known fatty acids are present in any quantity. Ether added to this solution readily dissolves the whole of the precipitate. Acetic acid alone, without any Na Cl added to original solution, produces a flocculent precipitate, which cannot be filtered clear, and when half filtered clogs the paper. From this mixture the precipitate can apparently not readily be extracted by ether. The watery fluid remains turbid, and the ether colourless. Hydrochloric acid causes an immediate curdy precipitate in the soapy mixture ; on boiling the precipitate does not fuse or coalesce, although it contracts it remains flaky. Some of the precipitate was shaken up with cold absolute alcohol, when much of it readily dissolved, and the filtered solution gave pulverulent precipitates with baryta water, and lead acetate. The entire quantity of the soap was now treated with hydrochloric acid, until a strongly acid reaction was attained, when a whitish yellow precipitate fell, and the fluid lost its viscosity. Filtration through Swedish paper was effected ; the precipitate on filter was washed with water, and the filtrate was evaporated on water bath.

Precipitation by H Cl.

1. *The Precipitate of Fatty Acids on the Filter* was washed, and became very adhesive yet fatty; boiled in water it did not fuse, but agglutinised a little ; nothing like oil or fused fatty acid appeared. Warmed on paper it did not fuse like fat, and gave only a very slight grease stain ; most of it remained slightly glistening on the surface, even when heated until brown. The matter had a fine, smooth, greasy touch between the fingers, and seemed like a fatty acid in a hydrated swelled state. It dissolved easily in cold absolute alcohol, leaving a quantity of adhesive dark matter undissolved. As it was treated on the filter, two litres of absolute alcohol were requisite to take up all soluble matters.

The acids.

2. *The Acids soluble in Alcohol.*—The solution showed a feeble green fluorescence ; it was filtered from a slight secondary deposit of insoluble acid. Alcoholic acetate of lead was now added, which produced a bulky nearly white precipitate. This was washed with absolute alcohol, drained on paper, dried, and treated with ether. It was entirely insoluble in ether, and constitutes

Acids soluble in alcohol.

a. Kephalophosphate of lead, so named from being a phosphorized acid obtained from kephaline, more complicated than glycerophosphoric acid. The total quantity of salt obtained weighed 6·2 grms. Powdered and dried at 95° C, it baked together a little on surface, but when stirred remained pulverulent.

Kephalophosphate of lead.

Determination of Lead.—0·6534 grm. carefully ignited, during which salt swelled much, became black, and evolved inflammable gases, left 0·2214 grm. white residue, apparently free from oxyde and metallic lead, and insoluble in acetic acid ; soluble in nitric acid, and containing phosphoric acid. This acid is, therefore, a compound of glycerophosphoric and another acid, and would, perhaps, by continued chemolysis with excess of free alkali, split up into those acids. The pyrophosphate found corresponds to 0·1558 grm., lead equal to 23·84% Pb in the salt. This leads to 661 as the atomic weight of the new acid (H_2 not added), and deducting 172 for supposed glycerophosphoric acid, to 489 as atomic weight of additional organic body.

C and H Determinations.—Substance dried at 80° C. 0·4144 grm. gave 0·7330 grm. CO_2 = 48·262 % C, and 0·2990 grm. H_2O = 7·990 % H.

A combustion for nitrogen gave only a trace of permanent gas, so that the substance is proved to be free from nitrogen.

Phosphorus calculated from the Pb pyrophosphate found = 3·572 °/₀ P.

	Found in 100.	÷ by At. Wgts	÷ by P = 1.
C	48·262	4·022	35·3
H	7·990	7·990	70·1
Pb	23·840	0·1151	1·01
P	3·572	0·1139	1·00
O	16·336	1·021	8·96
	100·000		

leading to formula $C_{35} H_{70} Pb P O_9$.

Deducting the lead, and calculating the 76·16 °/₀ of organic matter as 100, we get—

		÷ by At. Wgts.	÷ by P as 1.	At. Wgts.
C	63·369	5.2807	34·9	420
H	10·491	10·4910	69·3 + 2	72
P	4·691	0·1512	1·0	31
O	21·449	1·3405	8·8	144
	100·000			667

The atomic weight derived from the Pb pyrophosphate is 663, which closely approximates the quantity found directly.

Summar

The conclusion drawn from this experience was, that chemolysis, with an amount of alkali insufficient to satisfy at least three molecules of acid, developed from one molecule of kephaline, must stop short of complete decomposition, and produce intermediate products, of which the principal one is kephalophosphoric acid. It is probably an acid containing three acid nuclei:

Kephalic, Oxykephalic } Glycerophosphoric =Kephalophosphoric.

But no nitrogenised nucleus, and by further chemolysis may split up into the acids obtained by its side.

Acids in alcohol solution.

b. The Alcoholic Solution, from which kephalophosphate has been precipitated by lead acetate, was distilled to about 300 c.c. This liquor, on cooling and standing, deposited an oil, which was isolated, and found to float on water, but on boiling it became *viscous and solid in the hot*, and the water became white, as if the acid were emulged in it. There is, therefore, here no oleic or other fusible fatty acid, but one or several new acids, which had not combined with lead in the acid solution, as no alkali had been employed to neutralise the acids before the addition of the first lead acetate. Oil and liquid were treated with ammonia to strong alkalinity, whereby a white emulsive solution was produced. To this water and watery acetate of lead were added, until, and as long as, a precipitate was produced. This was white, adhesive, and bulky. It was filtered, washed, and dried, powdered, dissolved in ether, filtered, decomposed with hydrochloric acid and water, the ether solution was washed, filtered, and when clear distilled to small bulk.

Lead salt produced.

The red solution was treated with watery ammonia, filtered, baryum chloride added, when a bulky precipitate was obtained. This was washed for a long time, until washings were nearly free from baryta, the precipitate was dried in air, and treated with ether; a coloured salt dissolved, and a white salt remained undissolved.

Transformed into baryta salt.

α. Coloured salt, soluble in ether = Oxykephalate of baryum.

Two salts separated by ether. Baryum, oxykephalate, &c.

The oxykephalate of baryum dissolves rapidly and abundantly in ether, and is precipitated from this solution by absolute alcohol. It is always of a dark colour, which intensifies in the ether, probably by oxydation. The salt is insoluble, or but little soluble, in boiling alcohol, and is not deposited from this solution in crystals as oleate of baryum is. The name of the acid is taken from the fact that while similar in composition to kephalic acid, it contains more oxygen than that acid. It cannot be decolorised by animal charcoal, either when in the state of sodium salt, or when in the free state dissolved in boiling alcohol, and all operations seem only to assist in furthering its oxydation. It seems that its radical, while in the kephaline, is the principal cause of the assumption of colour and fluorescence by that principle.

β. White salt, insoluble in ether = Kephalate of baryum.

Properties of free acid.

The kephalate of baryum appears very much swelled in ether; when dry it is white and pulverulent. Hydrochloric acid and water extract the baryum, and washing with water leaves the acid soluble in ether; the ether distilled off leaves the acid as a coloured soft mass, which fuses at 26°, congeals at 25°, and crystallises in rosettes like margaric acid. It dissolves easily in absolute alcohol, forming colourless fluid; a few drops of water added to alcohol cause acid to separate as an oil on top of spirit; on standing, rosettes of crystals, colourless, form in this oil, and may be separated. The spirit deposits white clouds of the acid.

Differences from oleic acid

This acid differs from oleic by its baryum salt being entirely insoluble in boiling alcohol. It is not fluid at ordinary temperatures, but semi-solid, and fuses only at 26°, and from concentrated spirit it is entirely separated and crystallises, while oleic acid remains dissolved in such spirit, and does not crystallise at such temperatures.

In the manipulation of these acids in the presence of absolute alcohol the formation of ethylic ethers, which easily ensues, has to be carefully avoided.

3. *The Acid insoluble in Alcohol.*—On exposure to the air it assumed a very dark colour, apparently from attraction of oxygen or moisture. The whole easily dissolved in ether; the solution had a dark red colour, and exhibited a powerful green fluorescence. Addition of two or three volumes of absolute alcohol produced a slight opalescence, but no precipitate, which seems to exclude kephaline. This was further confirmed by the fact that lead acetate produced no precipitate in the mixture, consequently the lead salt, if any be formed, is soluble in ether, as well as ether alcohol, in which latter lead-kephaline is insoluble.

The whole of the acid was dissolved in ether, neutralised with ammonia, Ba Cl_2 added, and the mixture shaken; it was washed with water, filtered from a small quantity of insoluble precipitate, and the solution mixed with an equal volume of alcohol. A baryum salt was precipitated, but imperfectly, and the precipitate was isolated and dried. It was small in quantity, much coloured, and was not analysed. It is probably an intermediate product of chemolysis.

Acid filtrate.

4. *The Acid Filtrate* containing that phosphorus which was not combined with the kephalophosphoric acid, in the form of glycero-phosphoric acid; some nitrogen in an unknown form; the sodium chloride

together with some free hydrochloric acid, was evaporated to a low bulk and its acidity carefully neutralised by caustic soda. Acetate of lead was now added as long as a precipitate was produced, and the white deposit was filtered off. It was boiled, filtered, and washed with hot water, decomposed with H_2S, the filtrate neutralised with calcic carbonate and lime water, filtered and evaporated. When concentrated the solution deposited crystalline *calcium glycerophosphate*.

Lead glycerophosphate.

Calcic glycerophosphate.

5. The solution from which glycerophosphoric acid had thus been removed was freed from lead by H_2S, the acetate was decomposed by repeated addition of hydrochloric acid, and evaporation to dryness, and the crystalline magma was treated with absolute alcohol to extract the hydrochlorate of the expected nitrogenised body. To this alcoholic solution $PtCl_4$ was added, when a slight yellow precipitate ensued, which was separated by filtration, washed with alcohol, dried. It could only represent a small portion of the nitrogen contained in the chemolysed kephaline; as the volatilised vapours had not been collected some volatile alkali may have escaped unnoticed. The *alcoholic mother liquor* of this platinum precipitate, was freed from platinum by H_2S. Evaporated, it left a syrupy residue, this was again treated with little alcohol and $PtCl_4$ and a slight deposit removed. The liquor on addition of much ether deposited an *oily body*, soluble in absolute alcohol re-precipitated by ether. Distilled with dilute H_2SO_4 and MnO_2, it gave a distillate which smelled of *acetic acid*, and after neutralisation by soda, was reduced by $AgNO_3$ and by $HgNO_3$, consequently contained *formic acid*. These may be considered as decomposition products of *glycerine*, or of a body *containing its radical*.

Oily $PtCl_4$ salt.

Summary of Results of first Chemolysis of Kephaline.—All first products are soluble, the soaps imperfectly, in water. Hydrochloric acid precipitates a mixture of four fatty acids.

Summary.

1. Kephalophosphoric acid, consisting probably of kephalic, oxykephalic, and glycerophosphoric acid, yet in combination, therefore imperfectly chemolysed.
2. Kephalic acid.
3. Oxykephalic acid.
4. Acid insoluble in alcohol (small quantity).
5. Glycerophosphoric acid.
6. Ammonia.
7. Glycerine.
8. A base giving oily $PtCl_4$ salt, and being perhaps glyceramine of a new type. It is most difficult to deal with, as its solubility in water, alcohol, as hydrochlorate and as $PtCl_4$ salt is very great, and its precipitation by ether by no means complete.

Second chemolysis.

Chemolysis of Kephaline, second Experiment by Soda.—40·7 grms. of pure kephaline, purified by the water solution, filtration and HCl process as before described, were mixed with 5 grms. of crystalline soda hydrate dissolved in 2 litres of water. The same phenomenon was observed during the boiling as in the first experiment. The boiling was continued during about 18 hours, of which 3 hours took place in water bath, and the other 15 hours on sand bath, the time taken to heat it to boiling on the three days during which experiment lasted not included. While on the first and second day the mixture had set into a jelly, it did after the last boiling not become gelatinous again, but remained a somewhat turbid thick fluid. To this HCl was added until the precipitate was curdy and the fluid strongly acid. The precipitate was separated by the filter. It could be washed but imperfectly, as after consolidation on the filter, the precipitate set into a gelatinous tremulous

Water bath.

Sand bath.

HCl.

Filtration.

Solution of acids in NH_4HO.

solid mass, which allowed no washing water to pass. This mass of acids was placed into a wide-mouthed bottle, shaken with water and treated with caustic ammonia. It formed a complete solution, which was filtered on hot funnel and left no trace of residue. The solution was opaque, and on agitation showed the silky clouds common to soaps. To this solution acetate of lead was added until the precipitate was curdy, and the liquid distinct and filterable. It was now passed through french filtering paper, and the precipitate washed with much water, being agitated constantly with a glass rod, so that all parts were well penetrated by water. When the filtrate was free from chlorine and from lead (from excess of acetate), the precipitate was allowed to drain on paper and a cloth and dried. It took many days to dry, being frequently crushed and stirred.

Acetate of lead precipitate.

Filtration and washing.

Ether extracted a colored body, and left a white salt insoluble. This was separated by filtration, and washed by being twice removed from filter, and shaken in a bottle with ether. Filtration was always difficult owing to the finely divided state of the salt.

Ether extraction.

The white Pb salt insoluble in ether was dried in the vacuum, and yielded, on preliminary analysis, results which showed it to be impure.

White salt insoluble in ether.

It was further transformed into baryum salt by H Cl, water, and ether, solution of acid in $N H_4 H O$, and precipitation by $Ba Cl_2$. The salt was extracted by boiling alcohol, and what remained insoluble was reserved for analysis.

The coloured Pb Salt soluble in Ether was red in transmitted light, and fluoresced green, altogether appeared like a solution of kephaline. It was concentrated by distillation, and the solution precipitated by absolute alcohol; a viscous salt fell down, which became hard in alcohol, was drained and dried in vacuo. After preliminary testing, it was transformed into Ba salt by H Cl, water and ether; solution of acid in $N H_4 H O$ and precipitation with $Ba Cl_2$; solution in ether and precipitation by alcohol.

Salt soluble in ether.

These salts, therefore, correspond to the *kephalate* and *oxykephalate* of the first chemolysis. Their further study is reserved for future opportunities.

Transformed into baryum salt.

Baryta water added.

Treatment of the Filtrate containing Glycerophosphoric Acid and Ammonium Base.—It was neutralised with baryta water, the excess of reaction being allowed towards alkalinity. A pure saturated solution of *lead chloride* in water was now added, which produced a flocculent white precipitate of glycerophosphate. The advantages of the lead chloride were, that no new acid was introduced into the fluid, from which the ammonium base had yet to be extracted. The fluid became slightly acid, and was corrected by baryta water cautiously added. When all was precipitated the *glycerophosphate of lead* was filtered off, washed, and dried. The mother liquor was concentrated, and then gave another not inconsiderable precipitate with lead chloride solution. The reaction was continued until a filtered sample of liquid remained clear with the chloride, and the second precipitate was isolated and united with the primary one. The previously feebly alkaline liquid was now feebly acid; it was evaporated to a low bulk and then to dryness. It was now dissolved in a minimum of water, and precipitated with absolute alcohol, until this reagent produced no further turbidity and no deposit on standing 12 hours. The alcoholic extracts were united, concentrated, and precipitated with $Pt Cl_4$. A yellow precipitate fell, which will be described further on. The matter insoluble in alcohol (Na Cl, $Ba Cl_2$, and some undefined organic matter), re-dissolved in water, and again treated with $Pb Cl_2$ solution gave a third precipitate of glycerophosphate, which was added to the other quantities. This

Lead chloride added.

Glycerophosphate removed as lead salt.

Liquid evaporated and extracted with alcohol.

$Pt Cl^4$ precipitate.

Third precipitate of Pb glycerophosphate.

process shows how difficult it is to remove completely glycerophosphoric acid from these solutions. They require repeated concentration and neutralisation, and whatever the lead salt employed the supposed insolubility of the glycerophosphate of lead is not sufficiently great in these fluids to cause the whole of it to fall down.

The glycerophosphate of lead dried to a hard slightly colored mass. (This hardening of glycerophosphates is also observed upon baryum salt, and the condition must be borne in mind when it is subsequently intended to decompose these salts.) It was repeatedly crushed, ultimately powdered and dried in water oven. The total weighed 7·4435 grm. Now assuming the 40·7 gms. kephaline to have contained (at 4·2% P) 1·7 grm. phosphorus, then this corresponds to 19·38 grms. glycerophosphate of lead, of which theory is :—

Acid.		Pb salt.	Pb pyrophosphate.
3 C	36	36	—
9 H	9	7	—
P	31	31	2 P 62
6 O	96	96	7 O 112
	172	Pb 207	2 Pb 414
		377	588

But as only 7·4435 grms. were obtained there is a deficiency of 11·9365 grms.

Preliminary analysis.

In order to ascertain approximately the purity of the lead glycerophosphate, 0·9110 grm. was burned until white, and left 0·7440 grm. $Pb_2P_2O_7$ while theory required 0·7104. The white residue was treated with acetic acid and found insoluble in it, which corresponds with pyrophosphate.

Transformed into calcium salt.

The lead salt was now decomposed with hydrothion, the filtrate treated with CO_2 in the cold to expel H_2S, and then with milk of lime to alkalinity, filtered and treated with some CO_2. The filtrate after 12 hours standing was evaporated near the boiling point, and filtered hot from the white precipitate of calcic glycerophosphate. This when dry weighed only 0·727 grm. Of this 0·3400, after strong ignition with HNO_3, left 0·207 grm. residue, equal to 60·8 % $Ca_2P_2O_7$. Pure glycerophosphate should leave 60·5% of pyrophosphate.

Acid Glycerophosphate of Calcium.—The aqueous filtrate from which the above salt had been removed whilst hot, was mixed with three volumes of alcohol, when a light bulky precipitate ensued. This was isolated by filtration, washed with alcohol and dried. It weighed 0·526 grm. Of this 0·2446 grm. gave 0·1250 grm. pyrophosphate, equal to 51·1 % residue. This corresponds to a salt which might be obtained from acid glycerophosphate of calcium by combustion.

$C_3H_7\ Ca\ P\ O_6$
$C_3H_9\ \ \ \ P\ O_6$

$C_6H_{16}CaP_2O_{12}$ = acid glycerophosphate of calcium.

This on combustion may lose—

$C_6H_{14}\ \ \ \ O_5$, and leave

$H_2\ Ca\ P_2O_7$, or half saturated acid pyrophosphate of calcium, or a body isomeric with it. By this theory 51·4 % residue should be left, which differs but little from 51·1 % found.

The quantity of glycerophosphoric acid lost in the process of transformation is enormous, and this is invariably observed in all experiments which I have made. This experience, and that of the hardening of the lead salt from brain matters, which is not observed upon synthetically prepared lead salt, and the peculiar hardening and fusion of the baryta salt from brain, which reminds of the bearing of kryptophanates and paraphanates from urine, and kreatylates from flesh, call for a renewed study of this acid.

Platinic chloride precipitate.

The Platinic Chloride Precipitate. — The yellow precipitate was washed with absolute alcohol. On drying it became horny, crumbled up, was viscous outside and discoloured, and adhered strongly to the paper. Water restored yellow colour and pulverulence. It dissolved in hot water, of which much was required. On cooling brilliant small crystals were deposited, amounting to 0·9428 grm. This by analysis proved to be almost pure ammonium salt, containing only a trace of potassium, which raised the residue left on combustion to 45·56 %, while 44·36 % are required by pure NH_4 salt. By evaporation over H_2SO_4 two further small crops of ammonium salt were obtained; the rest of the solution dried up to a thick liquid *and did not crystallise.*

The filtrate from the $PtCl_4$ salt was mixed with a large excess of ether until this produced no further turbidity. An *oily matter* settled, which was purified by repeated solution in alcohol and precipitation by ether. It was not analysed, as there were no guarantees of its freedom from free glycerine. It was distilled with H_2SO_4 and MnO_2, and yielded an acid distillate, in which, after neutralisation with soda, the presence of *formic acid* was signalised by the usual tests.

This platinum salt which from the reactions detailed may split up into glycerine and ammonia, reduces the platinum rapidly when left in contact with ether alcohol, and becomes black. Altogether this product is one of the most difficult matters to treat, and particularly as it is only obtained in small quantities.

Third Chemolysis of Kephaline, with Baryta Hydrate. — The kephaline was prepared by the HCl process and well washed with water. The quantity used was 27·8 grms. It was placed in a flask connected with a condenser, and a solution containing 80 grms. BaH_2O_2, $10H_2O$ was added. The mixture was boiled during five hours on a sand bath, when the precipitate became adhesive and flask cracked. After cooling and filtering the residue was twice boiled with water, during which it became quite soft and semifluid; it was again thrown on a filter and washed to neutrality. The washings were united with first filtrate.

The baryum salts.

The baryum salts being drained of water were rubbed in a mortar with ether, and then shaken in a bottle with much ether. The ether *dissolved a colored salt*, leaving a *white salt undissolved*, from which solution was separated by filtration.

The insoluble white salt was dried in air; later in vacuo. It had the appearance of kephalate, and was reserved for future analysis.

The soluble colored salt had the appearance of oxykephalate, was red and fluoresced green. Solution was concentrated and precipitated by absolute alcohol. The precipitate was dried in vacuo and reserved for future analysis.

Baryum glycerophosphate.

The liquid containing the glycerophosphate and expected ammonium base was treated with carbonic acid until no more precipitate was produced, filtered, and filtrate concentrated on a water bath nearly to dryness. During this evaporation it did not deposit any salt, as does lime salt. The viscous mass was diluted with a little water to fluidity, placed in a bottle, and mixed with absolute alcohol until no further precipi-

tate was produced. The precipitate was filtered off and washed with alcohol.

The precipitate consisted of baryum glycerophosphate. It was bulky, white, and granular, probably alcoholate-hydrate. On standing, it contracted, and became horny, transparent, and partly fused. Placed in a glass dish, it fused entirely in a few days, and then dried to a brittle mass.

Summary of Analyses.

C	12·611
H	2·933
Ba	40·950
P	9·266
O	34·240
	100·000

Formula.—$C_3 H_7 Ba P O_6, H_2 O$.

Alcoholic solution.

The alcoholic solution from which the glycerophosphate had been removed was distilled to near dryness, and placed in a vacuum to crystallise. A few rosettes of crystals formed, the rest remained syrupy and alkaline. The crystals were white rhombic, spear-shaped, under microscope. In sublimation tube, they blackened, did not sublimate at all, fused, gave only stinking distillates, which were heavy, and did not crystallise at all (against sarkosine and alanine). The solution in water has a slightly acid reaction, so they are not solutions of an alkaloid or an ammonium base. Quantity too small for further study.

Last mother liquor.

The mother liquor, evaporated to a syrup, gave a few secondary crystals, too minute for isolation. The strongly alkaline syrup was soluble in water, less in alcohol, insoluble in ether. $H_2 S O_4$, $H N O_3$, and H Cl acids gave no precipitates in it. Acetate of lead gave a strong precipitate. $Hg Cl_2$, cold saturated solution, gave a strong precipitate. Phospho-molybdic acid gave a slight precipitate. $Pt Cl_4$ and a little H Cl gave a strong precipitate, which became adhesive and fused in air. Ether gave a strong precipitate in absolute alcohol solution. This was isolated, dissolved in absolute alcohol by the aid of heat, when a portion became insoluble, filtered and precipitated by much ether. Red fluid, oily body, becomes crystalline, on standing, in parts, reduced and brown in others. When isolated, is not entirely soluble in alcohol, therefore changes without intermission.

Kephaline and Lead.—The following preparation was made from a specimen which had not undergone the purifying process with water, filtration, and hydrochloric acid. It may, therefore, have contained some of the kephaline as ammonium salt. It had been thrice precipitated from ether by alcohol, and when last dissolved in ether, had stood during 24 hours in ice, to deposit the last traces of cerebrine.

Analyses (1.) 0·4622 grm., dried in vacuo, and ultimately at 90° to 92° until constant, gave 1·0086 grm. $C O_2 = 0·27507$ grm. C, or 59·51 % carbon, and 0·3877 grm. $H_2 O = 0·04307$ grm., or 9·31 % hydrogen.

(2.) 0·3748 grm. gave 9·1 c.c. N at 17·5° C., and 766 m.m. B., which, with corrections for potash column of 216 m.m. of 1·3 sp. gr., and other corrections, leads to 2·7 % nitrogen.

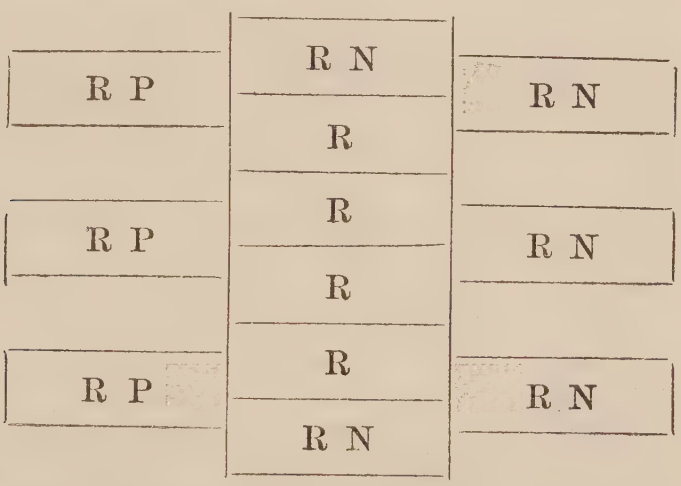
(3.) 0·6125 grm. fused with soda, nitre, and carbonate, &c., gave phosphate of magnesium = to 0·0223 P, or 3·64 % phosphorus.

Summary of Analysis of Free Kephaline—

		÷ by At. Wgts.	P as 1.	P=3.
C	59·51	4·96	42	127
H	9·31	9·31	79	238
N	2·73	0·195	1·6	4·98
P	3·64	0·117	1	3
O	24·81	1·550	13	39·7
	100·00			

Hypothesis regarding excess of nitrogen.

It will thus be seen that all the elements are in proportions required by pure kephaline, except the nitrogen, which is six tenths of an atom too high, or amounts to nearly five atoms, if three atoms of phosphorus are assumed. I do not admit any analytical fault, where it cannot be proved, simply because the results are anomalous. They must, therefore, be dealt with as such, and explained on the basis of the facts. Now, if as above surmised, any ammonia had been contained in the salt, two-thirds of the kephaline must have been so combined. Or if, as is probable from analogy with myeline, there are kephalines containing amidated acids, as proximate conjugated compounds, which amidated acids have also now and then been found in the chemolyses of kephaline, then it might be supposed that out of six fatty acid radicles in two molecules of kephaline, two fatty acid radicles were amidated, a condition represented by the following diagram, in which R signifies radical and the apposed letter P "containing phosphorus," and N "containing nitrogen"—



But the question is less foreclosed if the facts are expressed by the formula—

$$\left.\begin{matrix} C_{42}\,H_{80}\,N_2\,P\,O_{13} \\ C_{42}\,H_{79}\,N\;\;P\,O_{13} \\ C_{42}\,H_{80}\,N_2\,P\,O_{13} \end{matrix}\right\} = C_{126}\,H_{239}\,N_5\,P_3\,O_{39}$$

No sulphur. Made into salt.

The preparation was proved to be free from sulphur by special analysis. Some anomalies in details evidently do not yield to either hypothesis. The specimen of kephaline was therefore again frozen in ether, and deposited a vestige of white matter; the clear solution was then poured into absolute alcohol containing lead acetate; the precipitate was filtered, washed, suspended in absolute alcohol, warmed to 45° to 50°, filtered hot, to extract possible traces of cerebrine and soluble matters. No deposit occurred in this alcohol on cooling. The lead salt was dried in vacuo, powdered, and found highly electric; it was suspended in and extracted with ether.

Analyses (1.) 0·4817 grm. gave 0·7797 grm. CO_2, equal to 44·144 °/₀ C; and 0·2854 grm. H_2O, equal to 6·583°/₀ H.

(2.) 0·4326 grm. gave 4·25c.c. equal to 3·73 c.c. normal = to 1·07 °/₀ N.

(3.) The Pb and P were determined by fusing a portion of salt in soda and nitre mixture, dissolving in HNO_3, and determining lead as sulphide, and phosphorus as $Mg_2P_2O_7$.

0·9682 grm. dried at 100° gave 0·3115 grm. PbS equal to 27·86 °/₀ Pb.

The filtrate from PbS yielded 0·1030 $Mg_2P_2O_7$, equal to 2·97 °/₀ P.

(4.) 0·8013 grm. gave 0·2544 grm. PbS, equal to 27·49 °/₀ Pb. This, tested with HNO_3 and H_2SO_4 and ignited, left 0·3062 grm. $PbSO_4$ = to 26·10 °/₀ Pb. The filtrate from PbS yielded 0·09 $Mg_2P_2O_7$ = to 3·09 °/₀ P.

(5.) A third Pb determination by burning with H_2SO_4, and extracting phosphoric acid, gave 26·54 °/₀ Pb.

Summary of Analyses—

C	44·14				
H	6·583				
N	1·07				
Pb	27·86	27·49	26·10	26·54	Mean = 26·99
P	2·97	3·09			Mean = 3·03
O	18·25				
	100·00				

Computation of results.

Calculation shows that in this compound the lead does not stand in any stœchiometric relations to any one element. It is in excess by about one-fourth over the quantity it should be if one atom of kephaline were combined with one atom of lead. Deducting the lead, and calculating the organic matter, *i.e.*, 73·01 as 100 we get—

	$\overline{K}$ in Pb salt.	Free $\overline{K}$ before.	Pure $\overline{K}$ gives.	Theory requires.
C	60·5	59·51	60·00	60·28
H	9·1	9·31	9·39	9·44
N	1·46	2·73	1·68	1·67
P	4·06	3·64	4·27	3·70
O	25·02	24·81	24·66	24·88
	100·00	100·00		

The ether and lead treatment, the combination and purification thereby effected has therefore brought this kephaline much nearer to the composition of the purest. The nitrogen has fallen below the per-centage required, the phosphorus has risen a little. There if, therefore, advantage in combining kephaline with lead, with a view so again decomposing it, and thus purify the substance. But the advantage is probably less than that obtained by the water and hydrochloric acid treatment. Moreover, the treatment is expensive in substance and ether, and the compound is not in atomic proportions, but tends to be basic. We shall, lower down, meet with a compound of oxykephaline with lead, which actually seems to be a tetrabasic fully saturated compound. Probably, further experiments on the combination of kephaline with lead may teach the conditions under which it

will combine with this element in atomic proportions. It has this advantage over other bases, that it is less soluble in water, and therefore not liable to be deranged by moisture after being once combined with kephaline.

Oxykephaline with cadmic chloride, $C_{42}\ H_{79}\ N\ P\ O_{14}$, $Cd\ Cl_2$.—When the white matter (Ox) has been extracted with ether and the kephaline removed from the ether solution by precipitation with absolute alcohol, there remains a bulky solution containing all lecithine (?), much myeline and some kephaline, together with the cholesterine previously contained in the white matter. When to this solution $Cd\ Cl_2$ is added a voluminous precipitate ensues, which, after washing, yields to ether a quantity of coloured salt. This after concentration is precipitated by alcohol, and purified by repetition of this treatment. It was of interest to know the composition of this precipitate. (The composition of the insoluble precipitate is given under myeline and lecithine.)

Analyses—(1) 0·4547 grm. dried at 80° gave 0·8025 grm. $C\ O_2$, equal to 48·12 °/₀ C, and 0·309 grm. $H_2\ O$=to 7·55 °/₀ H.

(2) 0·5356 grm. gave 7c.c. N at 22° C. and 764 m.m. B. KHO column =22·4 m.m. Hg ; equal to 6·15c.c. N normal, or 1·43 °/₀ N.

(3) 0·9042 grm. fused with soda, nitre, and carbonate gave 0·2708 grm. $Ag\ Cl$ equal to 7·40 °/₀ Cl (these require to form $Cd\ Cl_2$ 11·66 Cd); and 0·1102 $Cd\ O$=11·65 °/₀ Cd ; and further 0·1142 $Mg_2\ P_2\ O_7$ equal to 3·524 °/₀ P.

Per cents. found.		÷ by At. Wts.		÷ Cd. as 1.	In 100 of organic matter.
C	48·12	81·95	4·01	42·21	58·71
H	7·55		7·55	79·47	9·21
N	1·43		0·102	1·07	1·74
P	3·524		0·113	1·18	4·30
O	21·33		1·33	14·00	26·02
					99·98
Cd	10·65	18·05	0·095	1.00	
Cl	7·40		0·208	2·18	
	100·004		100·00		

leading to formula $C_{42}\ H_{79}\ N\ P\ O_{14}$, $Cd\ Cl_2$.

Peculiar features of this salt.

This compound is noteworthy on account of two features, viz. : that it coincides with the composition of the theoretical $Cd\ Cl_2$ salt of kephaline, plus one atom of oxygen, and that the $Cd\ Cl_2$ is a complete molecule, combined with an apparently complete molecule of organic matter. Such compounds as this and the ones to be described hereafter with 15 atoms of oxygen, make one regret that there are no means of determining oxygen directly in organic chemistry. The oxygen is estimated by the void left by the substances determined, and this gives an opportunity for small impurities to be summed up under the guise of this element. Now the substance here considered had not undergone the process of purification by water, filtration, and acid, and it may therefore have been kephaline to which some slight impurity was attached. On the other hand there is no proof of the existence of such impurity and none could be found by testing. It is therefore necessary to consider this substance as a genuine compound of a kephaline containing an atom of O more than the normal kephaline, to which it will be convenient to apply the term *oxykephaline*. In any

case the isolation of this body from the mother liquor of kephaline by the ether process, by means of $Cd Cl_2$ is of sufficient importance in itself, no matter how the question of the atom of oxygen may ultimately be decided by further research.

Behaviour of a similar salt with water.

Behaviour of a similar Salt with Water.—A portion of a salt similarly obtained, though not analysed, was digested with water, whereupon it began to swell, and the water after filtration was found to contain large quantities of $Cd Cl_2$, proved expressly by the hydrothion and silver tests. When the extraction with water had been continued for some time filtration was effected, when during washing, the body swelled to such an extent as to clog the filter. There was therefore no guarantee of its purity from $Cd Cl_2$, except that it began to dissolve in water. The experiment proves that by simple digestion with water much $Cd Cl_2$ is extracted, but probably the entire amount can only be removed by a long *process of dialysis*. The results of experiments bearing in that direction will be described on a future occasion. Here it may be stated that the kephaline compounds are dialysed without a diaphragm, their own substance acting as dialysor in contact with water ; the diaphragm is only wanted to keep them from dissolving. The compound cannot be freed from cadmium by $H_2 S$, as when so treated in ether solution, it only assumes a yellow colour, and the Cd S remains dissolved. This peculiar bearing of many phosphorised compounds will also be fully described lower down. These, in themselves, highly remarkable peculiarities, throw many difficulties into the way of the extraction of the organic matter from the $Cd Cl_2$ precipitates, which are on the other hand so convenient for the removal of the organic matter from a highly complex mixture.

Dialysis necessary.

Peroxykephaline $C_{42} H_{79} N P O_{15}$.

How obtained.

A quantity of kephaline, which is described in my notes as having been obtained after frosting the ether solution and precipitating it by absolute alcohol, was subjected to elementary analysis, without having undergone the water filtration and HCl process.

Analyses.

Analyses.—(1) 0·4206 grm. dried at 80° C gave 0·8906 grm. $C O_2$ equal to 57·750 °/₀ C ; and 0·3370 grm. $H_2 O$ equal to 8·902 °/₀ H.

(2) 0·6160 grm. gave 8·9c.c. gas at 761 m.m. and 25 m.m. Hg potash column, equal to 7·75c.c. normal or 1·573 °/₀ N.

(3) 0·8190 grm. fused with caustic soda mixture, &c. gave 0·1080 $Mg_2 P_2 O_7$, equal to 3·68 °/₀ P.

Summary—

	In 100	÷ by At. Wts.	÷ by N=1.
C	57·750	4·8125	42·85
H	8·902	8·9020	79·26
N	1·573	0·1123	1·00
P	3·680	0·1187	1·05
O	28·095	1·7560	15·63

Formula.

Leading to formula $C_{42} H_{79} N P O_{15}$; At. W. = 868.

Transformed into lead salt.

Transformation of this Body into lead Salt.—About 10 grms. of the analysed substance were dissolved in ether, and to this solution *a warm solution of alcoholic lead acetate* was added. A viscous precipitate was produced, which settled in a mass. The lead acetate was not used in excess, the addition being discontinued while there was still a little matter in solution admitting of precipitation. The mother liquor was poured off, the mass of the precipitate was stirred and rinsed with a little ether first, and afterwards with absolute alcohol. The precipitate

Insoluble part.

was now digested with a quantity of ether, which dissolved much and left a portion undissolved. The insoluble part had a more gelatinous appearance than the original whole, and was disregarded. The solution was filtered off and precipitated by absolute alcohol, the precipitate was washed four or five times with absolute alcohol, dried and analysed.

Analyses.

Analyses of the Salt soluble in Ether.—(1) 0·830 grm. dried at 80° C, were fused with soda, nitre, and carbonate mixture, the lead precipitated by H_2S, and the Pb S transformed into Pb SO_4 by ignition with HNO_3 and H_2SO_4. The Pb SO_4 weighed 0·3872 grm. equal to 31·86 °/₀ lead. The portion of salt further gave 0·0808 grm. $Mg_2P_2O_7$ equal to 2·71 °/₀ P.

(2) 0·4255 grm. gave 0·5986 grm. CO_2, equal to 38·367 °/₀ C; and 0·2206 grm H_2O, equal to 5·760 °/₀ H.

(3) 0·5653 grm. gave 5·1 c.c. N at 25° C and 764 m.m. B. KHO column = 22 m.m. Hg, equal to 4·41 c.c. N normal or 0·9755 °/₀ N.

Summary—

	°/₀ found	$C_{42}H_{75}Pb_2NPO_{15}$ requires
C	38·367	39·436
H	5·760	5·868
N	0·9755	1·095
P	2·717	2·425
O	20·312	18·782
Pb	31·869	32·394
	100·000	100·000

It will be seen by a comparison of the oxygen quantities in the free body, with those of the salt, that there is no reason for assuming lead to be present as oxyde; on the contrary, the H being less in the organic part of the lead salt than in the free body justifies the assumption of a substitution of H_4 by Pb_2.

Comparison of results.

Comparison of the Organic Matter in the Lead Salt, with the Composition of the Free Body and of the Organic Matter in a Salt of Kephaloidine with Cd Cl_2.

	°/₀ found in original free peroxykephaline.	°/₀ found in Cd Cl2 salt of kephaloidine.	°/₀ found in lead salt of peroxykephaline.
C	57·75	57·91	56·31
H	8·90	8·82	8·30
N	1·57	1·67	1·43
P	3·68	3·71	3·98
O	28·09	27·78	29·81

The phosphorus, as in nearly all analyses, is found somewhat too high, rising in the lead salt to 5 as compared with N as 4. But on the whole, the change by the removal of some insoluble salt is not so great as to negative the assumption that the free body, and body contained in the lead salt have essentially the same composition, more particularly *the proportion of oxygen has not been decreased by the combination.*

Kephaloidine.

Why distinguished from

The substance thus designated is much like kephaline as above described, but presents some slight differences, which, though it may be

identical *in composition* with kephaline, imply the necessity of a preliminary distinction. It is obtained from buttery matter; kephaline from white matter; it is more fluid than kephaline when first precipitated, and never dries to the same hard brittle substance as kephaline, but presents a fused appearance. It presents the same irregularities in its combinations as kephaline; *it forms oxykephaloidine*, which like oxykephaline combines with a molecule of $Cd Cl_2$ forming a pretty concise salt.

Solubility in water and filtration.

Solubility in Water and Filtration.—Five grams of dry, hard, but viscous kephaloidine were dissolved in 500 c.c. of cold water with trituration. The substance became mucous at first, and on agitation in a long cylinder was disintegrated, and a turbid emulsion-like solution resulted. This was passed through the pressure filter, and passed easily 10 layers of English filtering paper. Next 10 layers of Swedish paper were employed, when an entire atmosphere of pressure allowed the liquid to pass but slowly.

Dialysis.

Bearing of Kephaloidine in Dialysing Apparatus.

First experiment.

First Experiment.—The foregoing solution after filtration, and subsequent treatment with animal charcoal, which did not make it clear, was distributed over two dialysers of parchment paper in gutta percha rings, the one six, the other five inches in diameter, and placed over 200 and 300 c.c. of water respectively. After 24 hours, the water gave but slight evidence of containing kephaloidine, by giving a mere vestige of precipitate with lead salt, while the original solution gave a very copious precipitate. Only a very minute portion of matter therefore had passed the diaphragm.

Second experiment.

Second Experiment.—A solution and emulsion of about 20 grms. of kephaloidine in 500 c.c. of water was, without having been filtered, subjected to dialysis, on three diaphragms. Each diaphragm after 24 hours had passed some kephaloidine, but only a very small quantity, as shown by the lead precipitate. The original solution on the dialyser gave a copious thick precipitate with the same lead salt. It was thus shown that a one per cent. solution can hardly be dialysed, while a four per cent. solution dialyses a little, but not enough for practical purposes. It may rather be expected that kephaline and kephaloidine act in watery solution, like colloids, and remain on dyalyser, while allowing the crystalloids mixed or combined with them to pass into the pure water. I have shown that they act as dialysors themselves when placed into pure water, and yield up the soluble salts or bases or acids with which they are combined. Probably dialysis by parchment paper may be found effective in completing this process.

May act like the colloids.

Bearing of the Ether Solution of Kephaloidine with Water.

Ether solution and water.

The kephaloidine was twice precipitated by alcohol from ether solution, and then redissolved in ether. A few drops falling in water are precipitated, and on shaking, a turbid emulsion is formed. Boiling transforms this into a turbid mucous mass with thick and viscid flakes. Hydrochloric acid added to this emulsion causes white curdling, and the white flakes can with difficulty be filtered off. The filtrate is white and turbid.

Water, hydrochloric acid, and ether in certain proportions produce a thick white jelly; a little more ether separates oily ether solution, which gives no precipitate with alcohol, or in this mixture with $Cd Cl_2$ and contains therefore H Cl.

Ether solution mixed with little water becomes a *solid white mass;* more water produces curds, ultimately emulsion and solution. A jelly thus produced with ether and much water deposited the ether solution on the surface. This with benzole, gave a white dense precipitate, re-soluble in ether and again precipitated by benzole.

Ether solution and little water.

Kephaloidine is easily soluble in benzole; treated with H Cl gas, this solution changes colour, but gives no precipitate; alcohol added to this gives a little precipitate, soluble in excess; ether gives no precipitate in the benzole H Cl solution. A solution of kephaloidine in anhydrous ether is not precipitated by benzole; the white dense precipitate described above requires the presence at the same time of water, HCl, ether, benzole, and kephaloidine. It seems to be a voluminous and stiff emulsion, which lies for days on blotting paper like jelly and ultimately dries to a thin film of kephaloidine.

Kephaloidine in benzole.

Reactions of the Watery Solution of Kephaloidine.

Hydrochloric acid produces a dense slightly yellow precipitate, which, after isolation, is soluble in ether and not precipitated by alcohol. In the filtrate from this precipitate $Pt\,Cl_4$ produces the merest opacity. But in the ethereal solution of H Cl precipitate, alcoholic $Pt\,Cl_4$ produces a precipitate soluble in ether and re-precipitated by alcohol.

H Cl.

Sulphuric acid produces a precipitate like that by H Cl. $H^2S\,O_4$.

Nitric acid, same as sulphuric. $H\,N\,O_3$.

Baryta water, Lime water, Cadmic chloride, Zinc chloride — All produce good precipitates which coalesce well on standing or agitation.

$Ba\,H^2\,O^2$. $Ca\,H^2\,O^2$. $Cd\,Cl_2$. $Zn\,Cl_2$.

Mercuric nitrate gives a good curdled precipitate insoluble in $H\,N\,O_3$, but made slightly yellow thereby. $Hg\,N\,O_3$.

Baryum chloride also produces a good flaky precipitate. This body after isolation is insoluble in water and absolute alcohol, but readily soluble in ether. $Ba\,Cl_2$.

Platinic chloride gives a complete precipitate. $Pt\,Cl_4$.

Lead acetate gives a good precipitate. $Pb\,(C_2\,H_3\,O_2)^2$.

Mercuric acetate gives a very voluminous precipitate. $Hg\,(C_2\,H_3\,O_2)^2$

Cupric acetate, a whitish flocculent precipitate. $Cu\,(C^2\,H^3\,O^2)^2$.

Kephaloidine lead.

Kephaloidine Lead.—A specimen of kephaloidine which had been frozen in the ethereal solution, and precipitated by alcohol, was once more dissolved in ether, and placed in a freezing mixture for 24 hours. No deposit occurred. This ether solution also gave the reactions above described, and with silver nitrate its watery solution gave a copious white precipitate. The ether solution was poured slowly in a thin stream into absolute alcohol, when the kephaloidine was precipitated as a viscid mass. The whole of this was dissolved in water, and lead acetate added; a copious precipitate ensued which was filtered and washed, extracted with warm dilute alcohol, ultimately with warm absolute alcohol; it then *dissolved in ether without residue;* was precipitated by absolute alcohol, became pulverulent, was dried in vacuo and analysed.

Analyses.

(1.) 0·9871 grm. dried at 70 to 75° C., later at 85° C., fused with caustic soda mixture, &c.; lead precipitated as Pb S and converted into $Pb\,S\,O_4$ gave 0·235 grm. $Pb\,S\,O_4$ equal to 16·26 °/₀ Pb, and 0·1296 $Mg_2\,P_2\,O_7$ equal to 3·666 °/₀ P.

(2.) 0·4579 grm. gave 0·8560 grm. CO_2 equal to 50·983 °/₀ C, and 0·3182 grm. H_2O, equal to 7·721 °/₀ H.

(3.) 0·6394 grm. dried at 100° c. gave 6 cc. N at 22° c. and 763 mm. B. KHO column = 21·4 mm. Hg; equal to 5·2 cc. normal, or 1·017 °/₀ N.

Summary.

	Summary.		In 100 organic matter.	Theory of $C_{42}H_{79}NPO_{13}$.
C	50·983	Total organic 83·740	60·88	60·28
H	7·721		9·22	9·44
N	1·017		1·21	1·67
P	3·666		4·37	3·70
O	20·353		24·32	24·885
Pb	16·260	16·260		
	100·000	100·000	100·00	100·000

It is at once evident that the Pb stands in no simple proportion to any other element. Kephaline lead if dibasic would require 19 °/₀, if monobasic 11 °/₀ of Pb. The molecule of the kephaloidinate contains thus rather more than half a molecule of Pb and is consequently a mixture of lead salt with free body. There is also an irrationality perceptible on the P which is too high, and the N which is too low. But on the whole the constitution, properties, and products coincide with those shown by kephaline of the compared formula.

Analyses.

Oxykephaloidine with cadmic chloride, 2 ($C_{42}H_{75}NPO_{14}$) + $CdCl_2$. In this case a quantity of kephaloidine obtained by the ether process, not purified by water, filtration, and HCl, &c., was transformed into $CdCl_2$ salt, and precipitated by alcohol; redissolved in ether, and the solution repeatedly frozen, and freed from some deposit, then precipitated by absolute alcohol. It could be dried at 80° C. without change.

Analyses.—(1.) 0·4694 grm. gave 0·9087 grm. CO_2, equal to 52·796°/₀ C, and 0·344 grm. H_2O, equal to 8·142°/₀ H.

(2.) 0·6396 grm. gave 8·75 c.c. N at 21·2° C. and 759 m.m. B; KHO column = 20·7 mm. Hg, equal to 7·69 c.c. normal, or 1·50°/₀ N.

(3.) 0·9368 grm, fused with caustic soda mixture, &c., gave 0·1340 grm. AgCl, equal to 3·53°/₀ Cl; filtrate neutralised with NH_4HO, and filtered from *slight* gelatinous precipitate, gave 0·1235 grm. $Mg_2P_2O_7$, equal to 3·68°/₀ P.

(4.) 0·9004 grm., fused as before, gave 0·1212 AgCl, equal to 3·33°/₀ Cl; further, 0·0430 grm. CdO, equal to 4·17 °/₀ Cd; the solution made alkaline, was not filtered this time, and gave 0·1198 grm. $Mg_2P_2O_7$, equal to 3·71 °/₀ P.

Mean of the two chlorine determinations 3·43°/₀ Cl.

This requires 5·41°/₀ Cd, so that there is a deficiency of Cd, probably lost in fusion by volatilisation of Cd.

Filtration of the alkaline solution before precipitation with magnesia mixture, diminishes P by 0·03°/₀, so that it is hardly necessary to effect this filtration of the frequently-observed slight clouds, for the difference would not affect the final result, and if, as is possible, the clouds were

phosphate of calcium, the phosphoric acid might be legitimate, and the calcium only an impurity of the nitre employed in fusion.

Summary :—

C	52·796	Total organic 91·160	
H	8·142		
N	1·500		
P	3·690		
O	25·032		
Cd	5·410		8·840
Cl	3·430		
	100·000		100·000

leading to formula $C_{42}\ H_{75}\ N\ P\ O_{14}$ for organic matter. If one atom of $Cd\ Cl_2$ were combined with two atoms of organic body, then 9·8% $Cd\ Cl_2$ should have been present.

Comparison of the Composition of the Organic Matter with

	Per cents of			
	Organic matter of oxykephaline with $Cd\ Cl^2$.	Organic matter in oxykephaloidine with $Cd\ Cl_2$.	Free peroxykephaline.	Organic matter in lead salt of peroxykephaline
C	58·71	57·91	57·750	56·31
H	9·21	8·82	8·902	8·30
N	1·74	1·64	1·573	1·43
P	4·30	4·04	3·680	3·98
O	26·02	27·45	28·095	29·81

It will thus be perceived that the oxykephaloidine is intermediate between oxykephaline and peroxykephaline in composition in all items except alone hydrogen. This peculiar anomaly, if such it be, must be reserved for future deliberation. The empirical formula expressing the composition of this salt is $2\ (C_{42}\ H_{75}\ N\ P\ O_{14}) + Cd\ Cl_2$.

The ethereal solution of this salt was not precipitated by hydrothion gas passed through it.

(*b.*). *Sub-Group of the Myelines.*

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Distinction.

General Definition of Myeline.—In selecting this name for a substance which has not been isolated before, I have to state that it must not be confounded with the matter so named by Virchow, and which is necessarily a mixture of several varieties of phosphorised principles, with several fats and cholesterine. The leading features of the principle here to be described will distinguish it with great precision from all similar matters. When freshly obtained it is white like bleached ivory, but on keeping becomes a little yellowish and waxy. It crystallises, from ether or absolute alcohol solution on slow evaporation, in curved needles and scales of a rhombic ovoid shape, which are well seen under the microscope with a power of × 400. When it is in minute crystals it remains powdery even after drying, but when drying in body after deposition from alcohol and washing by ether, it becomes transparent and waxy, cuts like dry walnut kernel, and when dry can be powdered. The powder is perfectly white. It emulges with water, and then dissolves, in the manner defined for all phosphorised cerebral principles. The solution iridesces bluish white from polarisation of the minute particles. This solution gives the reactions to be described. It dissolves in hot alcohol abundantly, and is deposed on cooling in white tufts, granules, and masses of peculiar appearance, and on slow evaporation in crystalline needles. The alcohol retains much myeline in solution when cold, and gives precipitates with $Cd Cl_2$ and $Pt Cl_4$. It dissolves in hot ether, and is almost immediately deposited from this solution when its temperature sinks. It is much less soluble in cold ether than in boiling. It contains more than 3% of phosphorus; gives with $Pt Cl_4$ a yellow salt, which is insoluble in ether, and soluble with some decomposition in boiling alcohol, from which it is deposited on cooling. It gives with $Cd Cl_2$ a white salt which is insoluble in ether, but soluble in boiling alcohol. With Pb acetate it gives a white salt, which is insoluble in alcohol and ether, and contains an atom of lead. Myeline is consequently a dibasic acid.

Leading features.

Colours.

Crystals.

Waxy solid.

Powder,

Emulges with water and dissolves.

Reactions.

Dissolves in hot alcohol, less in cold.

Dissolves in hot ether.

Contains P

$Pt Cl^4$ salt.

$Cd Cl_2$ salt.

Pb salt.

Modes of obtaining Myeline.—It can be obtained directly, without the intervention of precipitants, from the cold alcohol extracts of white matter, by concentration and cooling, redissolving the precipitate, and letting the solution stand for a long time in the cold, when myeline is deposed crystalline. After isolation to be washed with a little ether.

Direct method.

The ether extracts of white matter may be precipitated by alcohol, the precipitate emulged with water, treated with lead acetate, washed, and extracted with hot alcohol and ether in succession, when ultimately white myeline lead, of the formula to be given, remains insoluble in these agents. The lead salt decomposed by H_2S in water, and precipitate extracted with hot alcohol, yields white myeline.

By lead.

Lead salt decomposed.

All the mixed phosphorised bodies may be precipitated with $PtCl_4$ from alcohol solution, the precipitate washed and extracted with ether, this will remove kephaline and lecithine, and leave myeline hydrochlorate platinic-chloride, as a yellow body undissolved. The $PtCl_4$ salt must be moistened with ether, and agitated with much water, and washed as long as water extracts either $PtCl_4$ or HCl. Then the residue must be treated with H_2S in water, and washed again until the washings are pure. If they refuse to filter, the mixture must be taken off the filter, put into a bottle with water, and be shaken and triturated, and again filtered. At last the black mass is dried, and then extracted with boiling absolute alcohol. The alcohol on cooling and gradual concentration deposits white crystalline masses of pure myeline.

By $PtCl_4$.

$PtCl_4$ salt. How decomposed.

The $CdCl_2$ salt must also be treated with much water, which removes $CdCl_2$, and must then be treated with H_2S. This process is less convenient than that with Pb or $PtCl_4$. The $CdCl_2$ salt cannot conveniently be decomposed with H_2S in the presence of alcohol or ether, as in that conjunction compounds of myeline with cadmic sulphide are liable to be formed.

Decomposition of of $CdCl_2$ salt.

Differences and Separation from other Cerebral Principles.—Myeline can be separated from kephaline and kephaloidine and allied bodies by the operator using the peculiarity of its being *very little soluble in cold ether*, in which these bodies and their compounds are easily soluble. The solutions should always be exposed to a very powerful freezing mixture, and filtered through a filter and funnel surrounded with freezing mixture. From *lecithine* myeline can be separated by cold absolute alcohol, in which this body is more soluble than myeline ; also by cold ether, in which it is also more soluble than myeline. Freezing precipitates in all these cases the myeline more completely than ordinary temperatures.

Separation from kephaline series.

From lecithine series

Myeline can be separated from the principles of the *cerebrine* series by boiling ether, in which the cerebrines are almost insoluble. This separation can also be effected by much boiling alcohol, in which both are largely soluble, but the cerebrines are almost insoluble in cold alcohol, in which therefore myeline would remain dissolved on cooling more readily.

From cerebrines.

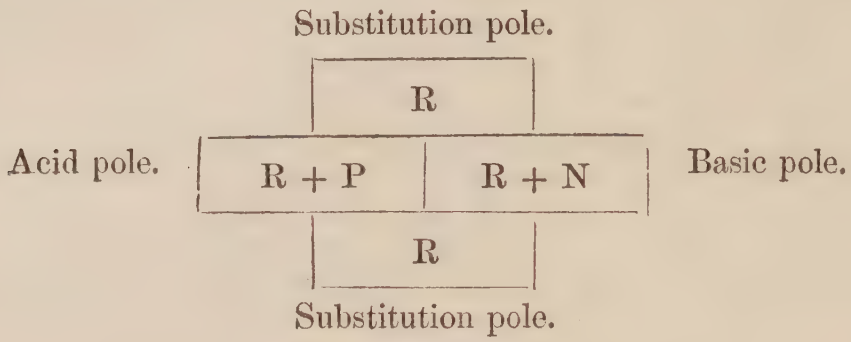
An absolute separation of myeline from the other phosphorised principles is best effected by the processes employing lead acetate and platinic chloride, and from cerebrines (as obtained by alcohol process) by extraction with boiling ether, or boiling alcohol and separation after cooling, or by cold benzole.

Absolute separation.

Probable Constitution of Myeline.—It contains four principal, or proximate radicals, of which one is the radical, compound itself, common to all the cerebral phosphorised substance, namely, glycerophosphoric acid. To this is attached a nitrogenised radical, choline or neurine, and

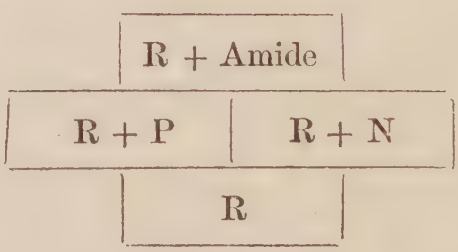
Constitution.

to these radicals are attached two fatty acid radicals, of varying value and composition, but most commonly consisting of margaric acid, with 16 C, or of the amidated form of this acid. Myeline combines with lead like a dibasic acid, with $Pt\,Cl_4$, and $Cd\,Cl_2$, like an ammonium base. From these facts we must attribute to its radicals positions which permit them to act alternately, if not together. These I have endeavoured to represent in the following diagram :—

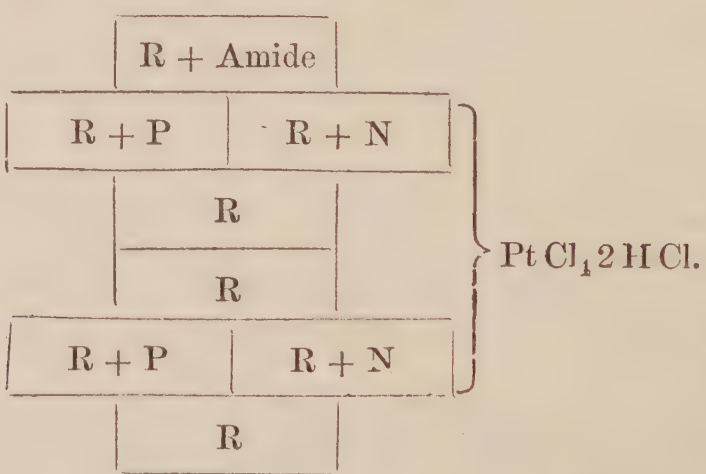


Constitution of amidated forms.

We find sometimes myelines which contain more nitrogen than phosphorus in the proportion of 2 : 1, or 3 : 2, or 5 : 3. In these cases I assume the increase of the nitrogen to be due to the presence of an amidated fatty acid radical, such as I have proved by chemolysis to occur in the rarer forms of kephaline. To such amidated forms I attribute the following constitution :—



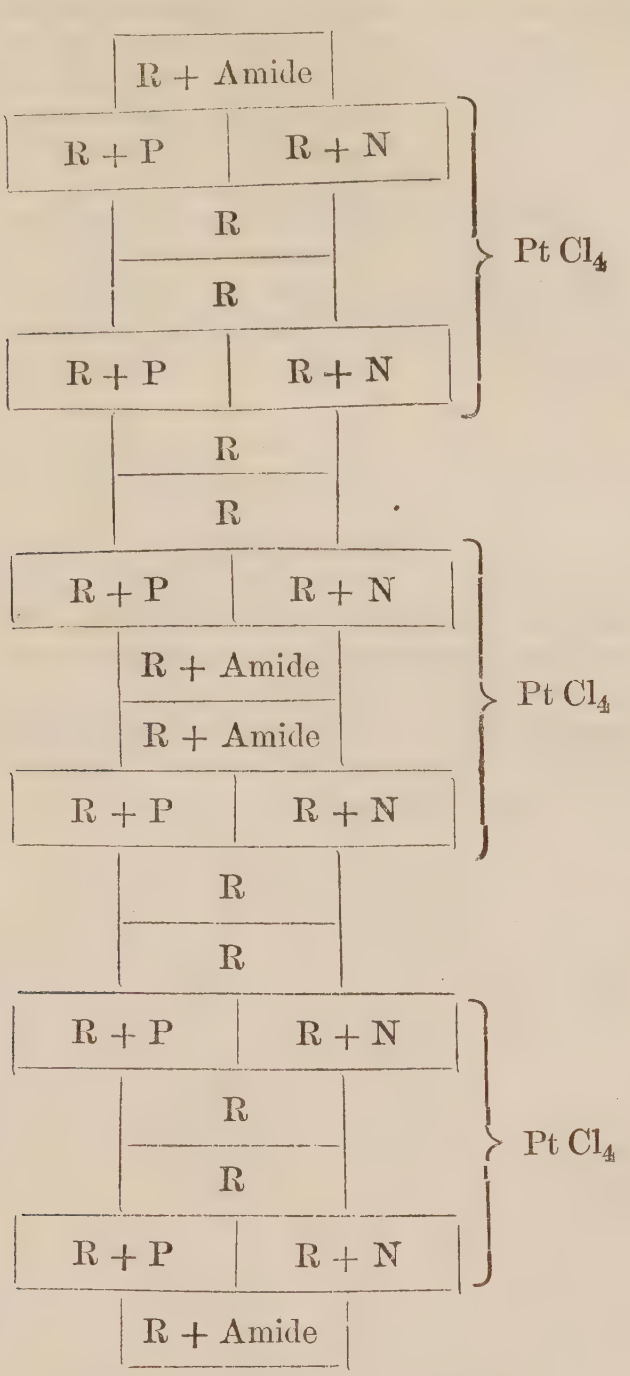
This most simple form I have never directly isolated, but I am compelled to assume its existence by the observation of salts, which could only be explained by any of the following schemes :—



Such a compound was sometimes united with $Pt\,Cl_4$ only, or with $Pt\,Cl_4$ and one H Cl, or with $Pt\,Cl_4$ and 2 H Cl, variations which made the preparation and analysis of these bodies extremely difficult and laborious.

But I have also met with compounds which contained when fully expressed six molecules of myeline, of which four were amidated (or two

twice amidated), and which require the following diagram for representation:—



I am yet doubtful whether the formula of myeline as above given must not be doubled, and whether the molecules are of equal value, or if isomeric are not somewhat differently constituted. At least myeline, like kephaline, yields to chemolysis products, in which the constituting radicals are presented in two different forms. The further study, confirmation, or refutation of this hypothesis I must reserve to the future.

Myeline-Lead.—C_{40} H_{73} Pb N P O_{10}. The ether-solution from white matter, after exhaustion by freezing, was precipitated by alcohol; the bulky precipitate was filtered, washed, and dried in vacuo, and during this process repeatedly pounded in a mortar. It was now dissolved in water, and subjected to fruitless dialysis; it formed a thick, slimy, gummy or starch-like emulsion, in which many small crystals formed like mica. The addition of watery Pb acetate produced a dense curd, which separated easily from fluid; it was placed on a cloth filter and allowed to drip over night. The precipitate was placed in alcohol and

Mode of obtaining.

Pb acetate added

Alcohol extractions.

Ether extraction

warmed, whereby little else but water was extracted (one litre alcohol left on evaporation to dryness a little brown matter). More warm strong alcohol now extracted much cholesterine and a little cerebrine Hot boiling absolute alcohol extracted *much* cholesterine and a little yellow smeary lead-salt. The insoluble part was soft, waxy, but on cooling granular. It was now placed in ether, whereby a yellowish fluorescent lead-salt of kephaline was extracted. This latter salt was precipitated by absolute alcohol, deposited as a yellowish oily body, which became hard on standing. This has been treated under kephaline.

Insoluble white salt left.

A white pulverulent salt remained insoluble in the ether, was thoroughly washed with ether on the filter, also shaken with ether in a bottle, and again washed on filter. It shrunk much on drying. It was insoluble in benzole.

Analyses.

Analyses: — Substance dried at 100° C 1) 0·4648 grm. gave 0·8672 grm. CO_2, equal to 50·88 % C, and 0·3304 grm. H_2O, equal to 7·89 % H.

2) 0·535 grm. gave 7 c.c. N at 21° C, & 761 m.m. B. K H O column = 19·5 m.m. Hg, equal to 6·18 c.c. normal or 1·44 % N.

(3.) 0·8961 grm. were fused with caustic soda, sodium carbonate, and nitre; the fuse was dissolved in HNO_3, nearly neutralised, and precipitated by H_2S. The Pb S was filtered off, dissolved in HNO_3, precipitated by H_2SO_4, and ignited. 0·2593 grm. $PbSO_4$ obtained, equal to 19·76% Pb. In the filtrate P_2O_5 was estimated by Mg mixture, and there was obtained 0·1056 grm. $Mg_2P_2O_7$, equal to 3·28% P.

Summary of Analyses and Theories:—

	Per-cents.	÷ by At. Wts.	÷ by Pb = 1.	÷ by N = 1.	÷ by P = 1.
C	50·88	4·240	44·63	41·56	40·38
H	7·89	7·890	83·05	77·35	75·13
Pb	19·76	0·095	1·00	0·93	0·90
N	1·44	0·102	1·07	1·00	0·97
P	3·28	0·105	1·10	1·02	1·
O	16·75	1·046	11·01	10·25	9·96
	100·00				

The organic body in the salt = 100 — 19·76 = 80·24.

Per-cents. of elements found in organic body and theories:—

		÷ by At. Wts.	by N = 1.	÷ by P = 1.
C	63·409	5·2840	41·24	40·09
H	9·833	9·8330	76·76	74·60
N	1·794	0·1281	1·	0·90
P	4·087	0·1318	1·02	1·
O	20·874	1·3046	10·18	9·89
	99·997			

There are thus arguments at hand for atomic weights with from 40 atoms to 44 atoms of carbon; but the combined metals and salts are under all circumstances less to be relied upon for atomic weight determinations of the phosphorised principles than the constitutional elements P and N. These latter, therefore, prevail in my opinion as determinants, particularly as they agree well with each other. I therefore

accept $C_{40} H_{73} N P O_{10}$ as the formula of the body combined with lead, and adding 2 H in place of Pb, the formula of the free body will be $C_{40} H_{75} N P O_{10}$.

	Theory of		Found.
Elements.	At. Wts.	Per-cents.	
40 C	480	63·15	63·409
75 H	75	9·86	9·833
1 N	14	1·84	1·794
1 P	31	4·07	4·087
10 O	160	21·05	20·874
	760		

Decomposition of Pb Salt by H_2S.—A portion was decomposed by $H_2 S$ while suspended in ether. The ethereal filtrate from the Pb S deposited a white flaky matter on being shaken, which increased in quantity on standing. It was allowed to go to dryness spontaneously, and left an abundant white residue, which was soft, and smelled peculiarly. It fused above 100, was perfectly fused about 125° to 130°, and on cooling was quite solid again at 100°. On being heated further it cracked and spirted, then gave off strong smelling fumes, burnt with a white luminous flame, and left a charcoal difficult to incinerate. Fused with nitre and soda, and the fuse dissolved in $H N O_3$, the tests for lead gave negative results, but the tests for $P_2 O_5$ gave evidence of abundance ; so that the $H_2 S$ treatment removed all the lead.

Pb S contains yet myeline.

The supposed Pb S, on heating, fused, and gave off carbonaceous vapours, and behaved in such a manner as to indicate that it did yet contain much organic matter. Therefore much ether, or better, boiling alcohol, will be required to extract all myeline from the Pb S.

The entire quantity of finely-powdered lead salt was now placed in absolute alcohol, and decomposed with $H_2 S$ while being heated in a water-bath, filtered hot, and extracted with hot alcohol. The alcohol extract, on cooling, deposited a crystalline mass. This was recrystallised from absolute alcohol, when a tendency to stearoconotise became evident in the deposit, but all ultimately dissolved with the aid of hot ether, and the first purest portion of crystals was analysed.

Analyses.—Dried at 100° C ; became coloured on surface.

(1.) 0·6756 grm. gave 0·1000 grm. $Mg_2 P_2 O_7$, equal to 4·17% P.

(2.) 0·4266 grm. gave 0·9800 grm. $C O_2$, = 62·651% C, and 0·3970 H_2O, = 10·340 % H.

(3.) 0·5000 grm. gave 9·c.c. gas at 760 m.m. B. and 20°5 C ; K H O=36 mm. Hg equal to 8 c.c. normal=2·00% N.

Summary and Computation :—

	Per-cents.	÷ by At. Wts.	÷ by P=1.
C	62·651	5·221	39·0
H	10·340	10·340	77·1
N	2·000	0·142	1·
P	4·170	0·134	1·
O	20·829	1·301	9·7

The isolated myeline thus exhibits the formula $C_{39} H_{77} N P O_9$, being the same as that of the non-amidated molecule in several $Pt Cl_4$ and $Cd Cl_2$ salts from ox and man, to be described later.

App. No. 5.
On the Chemical Constitution of the Brain, by Dr. Thudichum.

Myeline-Platinic Chloride, Modes of preparing.—A quantity of a mixture of myeline and lecithine from W. M. was placed in hot water and diffused and dissolved. The solution was filtered through force filter, acidified by H Cl and $Pt Cl_4$ added. The almost colourless gelatinous precipitate floated in the froth produced by shaking. The liquor was syphoned away from underneath the precipitate, and the latter thrown on a filter. On addition of a little water it immediately began to swell, and would not filter. It was therefore transferred to a beaker and agitated with alcohol of 85°/₀, whereby it became condensed. The liquid was again removed and absolute alcohol given to the precipitate and the mixture shaken. This caused a great condensation of the precipitate, which was thrown on a filter, washed with absolute alcohol, isolated, and somewhat dried. It was now placed in a considerable quantity of ether, as previous experiment had shown that if but little ether was employed there would be difficulty in filtration. A considerable quantity of a *yellow salt remained undissolved*, being the *compound of myeline and* $Pt Cl_4$, while the yellow filtrate contained the *lecithine salt*, which was isolated by precipitation with absolute alcohol.

Alcohol treatment.

Ether treatment.

Separation of salts.

Three further preparations.
Modified process.

Three further preparations were made with this modification of the process, that the precipitates as obtained by H Cl and $Pt Cl_4$ were, without being heated with water, immediately after separation from the first mother liquor agitated with an equal bulk of cold absolute alcohol and a few drops of $Pt Cl_4$; then washed with absolute alcohol, and then separated by ether. The insoluble precipitates from preparation 1 and 2 were united with each other, as were the insoluble ones from preparations 3 and 4, and analysed separately.

Analysis of Preparation 1 *and* 2.—After drying in vacuo lost 2·7°/₀ at 95° C.

(1.) 0·9027 grm. fused with caustic soda, sodium carbonate, and nitre, &c. left 0·05440 grm., equal to 6·026°/₀ Pt. Further, 0·1948 grm. Ag Cl giving 4·569°/₀ Cl. The Pt found requires 4·334 Cl, so that there is only 0·230°/₀ Cl. to be placed on account of any H Cl or, in other words, the compound is not a hydrochlorate. There were further obtained 0·1078 grm. $Mg_2 P_2 O_7$, corresponding to 3·334°/₀ P.

Summary :—

	Per-cents.	÷ At. Wts.	÷ by Pt=1.
Pt	6·026	0·0306	1
Cl	4·564	0·1285	4·19
P	3·334	0·1075	3·51

There was therefore an irrationality between the $Pt Cl_4$ and the phosphorus, showing that if two molecules of myeline were in combination with $Pt Cl_4$, one and a half molecule, or at least one molecule more of myeline must have been combined with this Pt salt. The body was herefore not any further analysed.

Analysis of Preparation 3 *and* 4.—

Pt
Cl
P.

(1.) 0·9311 grm. fused, &c. gave 0·0598 grm. Pt, equal to 6·442°/₀ Pt. Further, 0·2468 grm. Ag Cl = 6·551°/₀ Cl. Further, 0·1208 grm. $Mg_2 P_2 O_7$ = 3·619°/₀ P.

N
C.

(2.) 0·5680 grm. gave 10·6 c.c. gas at 13° C. and 777 mm. B K H O = 163 mm., equal to 10 c.c. normal = 2·202°/₀ N.

H.

(3.) 0·5120 grm. gave 1·066 $C O_2$=56·782°/₀ C and 0·447 $H_2 O$ = 9·992°/₀ H.

It is therefore evident that the Pt : Cl in this case is nearly as 1 : 6, but that both P and N stand in no direct ratio to $Pt Cl_4$.

Summary of Analyses :—

	Per-cents.		÷ by At. Wts.	÷ by Pt=1
C	56·782	87·007	4·7314	145·1
H	9·992		9·9921	—
N	2·202		0·1572	4·82
P	3·619		0·1167	3·58
O	14·412		0·9007	—
Pt	6·442	12·993	0·0326	1·
Cl	6·551		0·1845	5·66
	100·000			

Calculated Per-centage for Organic Molecule :—

	Per-cents.	÷ by At. Wts.	÷ by P=1.	P=3.
C	65·2666	5·4388	40·52	,,
H	11·4850	11·4850	85·58	,,
N	2·5310	0·1810	1·34	4·
P	4·1600	0·1342	1·00	3·
O	16·5574	1·0348	7·71	
	100·0000			

Disregarding small discrepancies a formula $C_{41} H_{85} N P O_8$ might be derived, but the nitrogen, as in other cases of first salts, is too high, being N : P = 4 : 3. If we assume three molecules of organic principle to be present, and one to contain an amidated radical, we obtain the following hypothesis :

$$\begin{array}{l} C_{40}\ H_{86}\ N_2\ P\ O_8 \\ C_{41}\ H_{85}\ N\ P\ O_8 \\ C_{41}\ H_{85}\ N\ P\ O_8 \\ \hline C_{122}\ H_{256}\ N_4\ P_3\ O_{24} \end{array}$$

On comparison of this myeline with that in the lead salt it will be seen that the hydrogen is by 10 atoms higher, and the oxygen by two atoms lower. The body in the Pb salt is therefore properly *oxy-myeline.*

Solution in and Recrystallisation from Boiling Alcohol. Formation of Apomyeline and Pt Cl_4

The foregoing analysed salt (prep. 1132, 3rd and 4th portion) was now heated with boiling alcohol, whereby most of it was dissolved and reprecipitated on cooling. The insoluble part was put aside. The recrystallised compound gave on analysis the following results :—

(1.) 0·4220 grm. gave 0·8430 grm. $C O_2$ = 54·481% C, and 0·3636 grm. $H_2 O$ = 9·573% H.

(2.) 0·5536 grm. gave 13 c.c. gas at 19° C, and 763 m.m. B. K H O = 16 m.m. Hg = 11·6 c.c. normal = 2·621% N.

(3.) 1·003 grm. heated by fusion method gave 0·0858 grm. Pt = ·8554% Pt. Further, 0·3662 grm. Ag Cl = 9·032% Cl, and 0·1260 grm. $Mg_2 P_2 O_7$ = 3·507% P.

App. No. 5.

On the Chemical Constitution of the Brain, by Dr. Thudichum.

Summary of Analyses :—

	Per-cents.	
C	54·481	
H	9·573	
N	2·621	
P	3·507	
O	12·232	
Pt	8·554	17·586
Cl	9·032	
	100·000	

Computation of Free Molecule :—

	Per-cents.	÷ by At. Weights.	by P = 1.
C	66·101	5·508	40·2
H	11·615	11·615	84·7
N	3·178	0·227	1·6
P	4·255	0·137	1·
O	14·837	0·927	6·7
	99·986		

The differences in the composition of the salts before and after solution in boiling alcohol are best seen in the following comparison.

	The Salt before Solution.		The Salt after Solution in Hot Alcohol.	
C	56·782		54·481	
H	9·992		9·573	
N	2·202		2·621	
P	3·619		3·507	
O	14·412		12·232	
Pt	6·442	12·993	8·554	17·586
Cl	6·551		9·032	
	100·000		100·000	

A remarkable increase in the per-centage of $PtCl_4$ becomes at once apparent; the other elements also change, but in a differing ratio, not wholly determined by the increase of the attached metallic salt. This change is perceived better upon the organic molecules, compared in their free state.

	Before.	After.	Apomyeline, Man.
C	65·2666	66·101	67·01
H	11·4850	11·615	11·35
N	2·5310	3·178	3·00
P	4·1600	4·255	3·23
O	16·5574	14·837	14·649
			+ Cl 0·761
	100·0000	100·000	

Carbon and hydrogen have slightly risen; nitrogen has risen by one-fifth of the quantity in the first salt, or from 5 to 6; phosphorus has

risen a little, and oxygen has greatly fallen. These changes seem to have reached a certain climax in the (human) apomyeline (from Pt Cl_4 salts, boiled in alcohol), which was examined in the free state. They indicate that myeline has perhaps a much higher atomic weight than that shown in the above empirical formula, and under the influence of acid and metallic salts in alcohol gives up certain proximate component inucle, containing P, while retaining all or most of its N.

Comparison of this body with Apomyeline (man.) $C_{52}\ H_{106}\ N_2\ P\ O_9$.— The theory of the Pt Cl_4 salt from ox (boiled), comes very close to this, except in the P.

Theory.			Found.
Atoms.		Per cents.	
104 C	1248	54·80	54·481
212 H	212	9.31	9·573
2 P		2·72	3·507
4 N		2·46	2·621
18 O			12·232
1 Pt		8·69	8·55
6 Cl		9·35	9·03

The relation of Pt Cl_4, Cl_2, and N_4, make this compound very important; if the difference in the P can be hereafter explained, it may perhaps be expressed by the formula—

$$\left.\begin{matrix} C_{52}\ H_{106}\ N_2\ P\ O_9\ Cl \\ C_{52}\ H_{106}\ N_2\ P\ O_9\ Cl \end{matrix}\right\} Pt\ Cl_4$$

Myeline-Platinic-Chloride from Ox. W.M. (preparation 1188). — This salt was prepared from the cold alcoholic extracts as above described, and was insoluble in ether. It was dried at from 70° to 80° C; the particles lost their light yellow colour, and adhered to each other, but were not otherwise altered.

Analyses:—(1.) 0·963 grm. fused with caustic soda, nitre, and potash carbonate, gave 0·0728 grm. Pt = 7·559 °/₀ Pt; further 0·3360 grm. Ag Cl = 8·630 °/₀ Cl; and 0·115 grm. $Mg_2\ P_2\ O_7$ = 3·334 °/₀ P.

(2.) 0·4716 grm. burned with chromate, &c., gave 0·8984 grm. $C\ O_2$ = 51·955 °/₀ C, and 0·380 grm. $H_2\ O$ = 8·952 °/₀ H.

(3.) 0·549 grm. gave 11·5 c.c. gas at 762 m.m. B and 20° C. KHO = 16·7 m.m. Hg, equal to 10·2 c.c. normal = 2·324 °/₀ N.

Summary of Analyses:—

C	51·955		
H	8·952		
N	2·324	83·811	
P	3·334		
O	17·246		
Pt	7·559	16·189	
Cl	8·630		
	100·000	100·000	

Computation of °/₀s of free organic body :—

	Per cents.	÷ At Weight.	÷ by P = 1.
C	61·990	5·166	40·3
H	10·681	10·681	83·4
N	2·772	0·198	1·5
P	3·978	0·128	1·0
O	20·579	1·286	10·0

leading to formula—

$$\left.\begin{matrix} C_{40}\ H_{83}\ N\ \ P\ O_{10} \\ C_{40}\ H_{82}\ N_2\ P\ O_{10} \end{matrix}\right\} = C_{80}\ H_{165}\ N_3\ P_2\ O_{20}.$$

H_2S treatment of Pt Cl_4 myeline.

Decomposition of the 1st and 2nd Insoluble Platinic Chloride Myeline Compound by H_2S, and Recovery of the Free Myeline in the Pure State.—This salt, the partial analysis of which has been described above, was triturated in a mortar with water to an impalpable powder, mixed with more water, and treated with H_2S in excess. Time was allowed for the complete action of the H_2S, and filtration was not effected until the mass had stood in a stoppered bottle for several days. The filtrate contained apparently no organic matter, and did not give the reactions of myeline. The washed and drained precipitate was now digested in boiling alcohol for some time, and filtered hot. The filtrate was slightly yellowish, and contained the free myeline as shown by the following tests :—

Reactions of alcohol solution.

On evaporation a portion left a white residue.
With water the alcohol solution became milky without giving precipitate.
Aqueous Pt Cl_4 gave an abundant yellow granular precipitate.
Pt Cl_4 + H Cl also gave the foregoing precipitate, but H Cl alone none.
Ba Cl_2 gave a good dense precipitate.
Ba H_2O_2 gave a somewhat gelatinous precipitate.
Aqueous Cd Cl_2 gave a bulky, but swelled precipitate.
Alcoholic Cd Cl_2 gave a good bulky precipitate.
Aqueous Zn Cl_2 gave a good white precipitate.

The bulk of the solution was now allowed to evaporate spontaneously, when it deposited minute *crystals, characteristic of myeline.* It was subsequently added to a similar preparation derived from the union of several Pt Cl_4 compounds, also decomposed by the H_2S process, and further studied as will be described.

Myeline-Platinic-Chloride from Ox Buttery Matter.—The buttery matter was dissolved in ether, and alcoholic Pt Cl_4 added. A yellow precipitate ensued, insoluble in ether, with which it was washed. Weight 4·7 grm., and gave on analysis the following results. Lost in air-bath at 70–80° C. 1·44 °/₀.

Analyses. Pt.

(1.) 1·2359 grm. were gradually introduced into a previously fused mixture of nitre and sodium carbonate. It was ascertained that in this manner some phosphorus and much chlorine were lost. Therefore the Pt alone was determined and found to be equal to 10·48 °/₀ Pt.

Pt. Cl.

(2). In this experiment 1·0484 grm. were boiled down with caustic soda leye, made from metallic sodium and nitre, in a platinum crucible, and heated to fusion, &c. There were obtained: Pt = 10·36 °/₀ ; Cl = 10·89 °/₀ ; P = 3·31 °/₀. We have therefore here almost exactly one atom of Pt to six atoms of Cl, *i.e.*, upon 197·4 Pt 213 Cl required, and 214·9 found.

(3.) 0·5933 grm. gave 1·0867 $C O_2$ = 49·94 °/₀ C and 0·4588 $H_2 O$, equal to 8·36 °/₀ H.

(4.) 0·7164 grm. gave 12·8 c.c. N gas, normal, equal to 2·23 °/₀ N.

(5.) 0·5722 grm. gave 11·755 c.c. N gas, normal = 2·57 °/₀ N.

Mean 2·4 °/₀ N.

Summary of Analyses :—

			÷ by At. Wt.	÷ by Pt = 1.
C	49·94		4·1658	79·1
H	8·36		,,	,,
N	2·40	78·69	0·1714	3·25
P	3·31		0·1067	2
O	14·68		,,	,,
Pt	10·42	21·31	0·0526	1
Cl	10·89		,,	6
	100·00			

C. And H. N. N.

Computation of Organic Molecule :—

	Per cents.	÷ by At. Wt.	÷ by P = 1.		M. Wt. P = 1
C	63·46	5·288	39·1	52·	468
H	10·624	10·624	78·7		77
N	3·05	0·217	1·6	2·1	14
P	4·20	0·135	1·		31
O	18·67	1·166	8·6		144
	100·004				734

The Pt Cl_4 + 2 H Cl is in excess of that molecule; 21·31 : 78·69 = 411 : 1517; $\frac{1517}{2}$ = 758·5. The nitrogen is too high for any simple formula, and stands to P = 3 : 2, or 5 : 3. We have therefore here also perhaps an amidated radical in one out of two molecules, and obtain as simplest hypothesis :—

$$\left.\begin{matrix} C_{39} H_{78} N_2 P O_9 \\ C_{39} H_{77} N P O_9 \end{matrix}\right\} + (HCl)_2 + Pt Cl_4.$$

Mycline-platinic chloride, $C_{78} H_{162} N_3 P_2 O_{16}$, Cl_2, Pt Cl_4, from ox buttery, preparation Nro. 1202.—This compound was insoluble in ether; soluble in benzole.

Analyses.

(1.) 0·9473 grm. dried at 60–75° C. were subjected to fusion, and yielded,—

(*a*) 0·089 grm. equal to 9·395 °/₀ Pt.
(*b*) 0·3840 grm. Ag Cl = 10·026 °/₀ Cl.
(*c*) 0·1164 grm. $Mg_2 P_2 O_7$ = 3·431 °/₀ P.

(2.) 0·4612 grm. gave 9·5 c.c. gas at 20° C. and 764 m.m. B. KHO column = 16·7 m.m. Hg = 8·5 c.c. gas normal, or 2·305 °/₀ N.

(3.) 0·4830 grm. gave 0·9062 grm. $C O_2$, equal to 51·168 °/₀ C; and 0·3900 grm., $H_2 O$ equal to 8·971 °/₀ H.

Summary of Analyses.

C	51·168	80·579
H	8·971	
N	2·305	
P	3·431	
O	14·704	
Pt	9·395	19·421
Cl	10·026	
	100·000	100·000

In this salt Pt : Cl = 1 : 6. But the Pt Cl_6 is about 2·5 per cent. too low when compared with the requirements of the computed free body. For the atomic weight of this 729 + 205·2 (being the molecule, with P = 1 + ½ molecule Pt Cl_6) requires 21·96 °/₀ of Pt Cl_6.

Computation of Organic Body free from Pt Cl_6.

	°/₀ s.	÷ by At. Wts.	÷ by P = 1.
C	63·500	5·291	38·6
H	11·133	11·133	81·2
N	2·865	0·204	1·489 (say 1·5)
P	4·257	0·137	1·0
O	18·245	1·140	8·3
	100·000		

These data lead necessarily to the empirical formula $C_{78}\,H_{162}\,N_3\,P_2\,O_{16}$ for the free substance, and with the aid of the hypothesis applied to other salts to formula—

$$\left.\begin{matrix} C_{39}\,H_{81}\,N\;\;P\,O_8\,Cl \\ C_{39}\,H_{82}\,N_2\,P\,O_8\,Cl \end{matrix}\right\} Pt\;Cl_4.$$

Stress must be laid upon the fact that here again N : P = 3 : 2.

Myeline Cadmic Chloride Salts from Ox Buttery.—The salts described in the following were all obtained from the buttery matter by the following process:—The matter was dissolved in ether, and precipitated by alcohol. No freezing or other artificial low temperature was employed. The solution, separated from the precipitate, was treated with Cd Cl_2 in alcohol; a voluminous white precipitate fell, which, after isolation and pressing, was exhausted with ether. The portion which remained *insoluble in ether* is the one here to be considered. It was soluble in boiling alcohol, and deposited from this solution on cooling perfectly white. It dissolved in hot benzol, and from this solution no deposit ensued on cooling. The fluid was red coloured, and had a green fluorescence. It was precipitated, whilst warm, with alcohol of 85 °/₀, filtered, washed with much alcohol, and pressed in a cloth. This process was repeated twice more with all formalities. The purified salt was next extracted with alcohol of 85 °/₀ at 45° C., by which a small quantity of salt was separated. The salt which was not dissolved is the one analysed below. (Further experience showed that absolute alcohol dissolved and re-deposited the whole of the compound.) As the product constituted the largest quantity of Cd Cl_2 salt obtained in this and similar processes, it was termed "principal cadmium salt, insoluble in ether."

Soluble in hot alcohol.

Solution in benzol and precipitation by spirit.

Analyses.

(1.) 0·9462 grm. fused with caustic soda and nitre, &c., yielded 0·3041 grm. Ag Cl, equal to 7·94 °/₀ Cl. and 0·1104 grm. $Mg_2 P_2 O_7$ equal to 3·26 °/₀ P.

(2.) 0·9500 grm. gave 0·2900 grm. Ag Cl = 7·55 °/₀ Cl, and 0·1132 grm. $Mg_2 P_2 O_7$ = 3·32 °/₀ P.

(3.) 0·8950 grm. fused, &c., and dissolved in dilute H Cl; Cd precipitated as Cd S: the latter dissolved in H Cl; the solution evaporated to drive off excess of acid, and then precipitated by $Na_2 C O_3$; precipitate ignited left 0·1288 grm. Cd O, equal to 12·59 °/₀ Cd.

Accidents.

It was found that the greatest care is necessary in this mode of analysis to prevent three kinds of accident—(1) volatilization of Cd; (2) formation of alloy of Cd with Pt; (3) excess of S in Cd S, which makes it impracticable to determine the Cd as sulphide.

(4.) 0·4186 grm. gave 0·7696 grm. $C O_2$ = 50·14 °/₀ C, and 0·3120 grm. $H_2 O$ = 8·28 °/₀ H.

(5.) 0·634 grm. gave 9·2 c.c. gas at 19° C. and 757 m.m. B. KHO= 16 m.m. Hg = 8·1 c.c. normal, or 1·59 °/₀ N.

Summary.

	Found in 100.	
C	50·14	79·47
H	8·28	
N	1·59	
P mean	3·29	
O	16·37	
Cl	7·94	20·53
Cd	12·59	
	100·00	

Computation of Percentage and Formula of Organic Molecule.

	°/₀	÷ by At. Wts.	÷ by P = 1.
C	63·09	5·257	39·52
H	10·41	10·41	78·27
N	2·00	0·142	1·06
P	4·13	0·133	1·
O	20·59	1·286	9·66
	100·00		

H_2S dissolves Cd Cl salt.

Behaviour of this Cd Cl_2 salt with $H_2 S$ in Ether.—A little of this salt was suspended in ether, and a few bubbles of hydrothion were passed through the mixture. *The precipitate immediately dissolved,* and a bright yellowish red solution resulted. It was thus evident that this peculiarity, which I had observed on many Cd Cl_2 precipitates before, was a general feature of these salts; *they could not be decomposed in ether by $H_2 S$,* because the Cd S formed passed into solution instead of being, as had been expected, precipitated. The solution was divided in three parts: in one alcohol threw down a yellow flaky precipitate, which readily subsided; in a second portion water threw down a gelatinous swollen mass, not unlike yelk of egg, which floated on the top of the solution; in a third portion ammonia behaved much like water, except that the whole solution became nearly solid. I have shown elsewhere that the yellow precipitate which alcohol produces in the ether solution contains the organic molecule of the Cd Cl_2 precipi-

No Cd S precipitated.

Alcohol precipitates yellow salt

tate, cadmium, chlorine, and sulphur. We may, therefore, consider that in the ethereal solution the compound behaves *like a mercaptan*, and being itself sulphurised for the time, has the power to combine with or hold in solution a metallic sulphide.

Similarity to mercaptans.

The salt, though insoluble in ether, is soluble in ether acidified with H Cl. In this solution, also, no precipitate is produced by $H_2 S$; but it assumes a yellow colour, and water produces in it a yellow gelatinous emulsion, which persists for weeks, if not treated with large volumes of ether. The yellow ether solution, on spontaneous evaporation, leaves a yellow residue behind.

Pt Cl4 applied to mother liquor of W. M.

Myeline Cadmic Chloride from Alcoholic Extracts of Human Brains after Removal of White Matter and Platinic Chloride Precipitates.—In some of my researches I applied $Pt Cl_4$ to the mother liquor of white matter in the hope of thereby effecting a precipitation of phosphorised bodies. But the results obtained from large quantities of material were insignificant. Much platinum remained in solution, and was partially recovered by the addition of ammonia. (The precipitate contained other matters, and in particular gave reactions of a metal not hitherto known in science.)

Distilled.

The entire solution was now concentrated by distillation, allowed to cool, and the deposited cholesterine removed.

Mixed with Aq.

The liquid was treated with a large volume of water, filtered, and the filtrate evaporated to about $\frac{1}{4}$ volume. A dark smeary deposit appeared in the evaporating dishes, which was added to the residue of the first filtration on filter.

Precipitates dissolved in ether.

These residues were dissolved in ether. This solution may in effect be considered as *a solution of buttery matter in ether, to which $Pt Cl_4$ has been added.*

$H_2 S$ treatment.

This solution was now treated with $H_2 S$ to remove $Pt Cl_4$, but this was only partially successful, for although a precipitate ensued, yet the fluid remained black, and retained Pt; it was therefore warmed on water bath, and $C O_2$ passed through it to drive off a large excess of $H_2 S$.

Alcohol precipitates several matters.

More than an equal volume of alcohol was now added to it, whereupon a dark brown bulky precipitate fell down; this was ascertained to contain kephaline, myeline, and lecithine, but the kephaline in prevailing amount. The ether alcohol filtrate contained the matter which gave the $Cd Cl_2$ salt to be described.

Cd Cl2 precipitates.

The solution was distilled to remove the ether, and the residue allowed to stand to deposit cholesterine. To the filtrate from this latter alcoholic $Cd Cl_2$ was added, whereby an abundant white precipitate was produced, which was washed with alcohol and dried. It was next extracted with alcohol at 45° C to remove any cerebrine. It was found to be insoluble in ether, but yielded to this solvent a quantity of matter, which was again precipitated by the addition of alcohol. (Kephaline and lecithine $Cd Cl_2$.) All these collateral precipitates were not any further investigated on account of the difficulty of freeing them from cerebrine and cholesterine.

$H_2 S$ dissolved precipitate in ether.

But the $Cd Cl_2$ precipitate, which remained insoluble in ether (though it dissolved in ether with the aid of $H_2 S$, and the solution gave a yellow precipitate of an organic Cd S compound with alcohol), was analysed with the following result.

Analyses.—(1.) 0·9660 grm. gave 0·3754 Ag = 9·61% Cl and 0·1122 $Mg_2 P_2 O_7$ = 3·24% P.

(2.) 0·7268 grm. gave 0·1426 grm. Cd S, equal to 15·25% Cd; 0·2827 grm. Ag Cl = 9·61% Cl; and 0·0820 grm. $Mg_2 P_2 O_7$ = 3·15% P.

(3.) 0·5576 grm. gave 0·979 grm. $C O_2$ = 47·88% C, and 0·3868 grm. $H_2 O$ = 7·71% H.

(4.) 0·5137 grm. gave 8·3 c.c. gas at 17·0 C and 762·5 m.m. B, &c., equal to 7·47 c.c. N normal = 1·81% N.

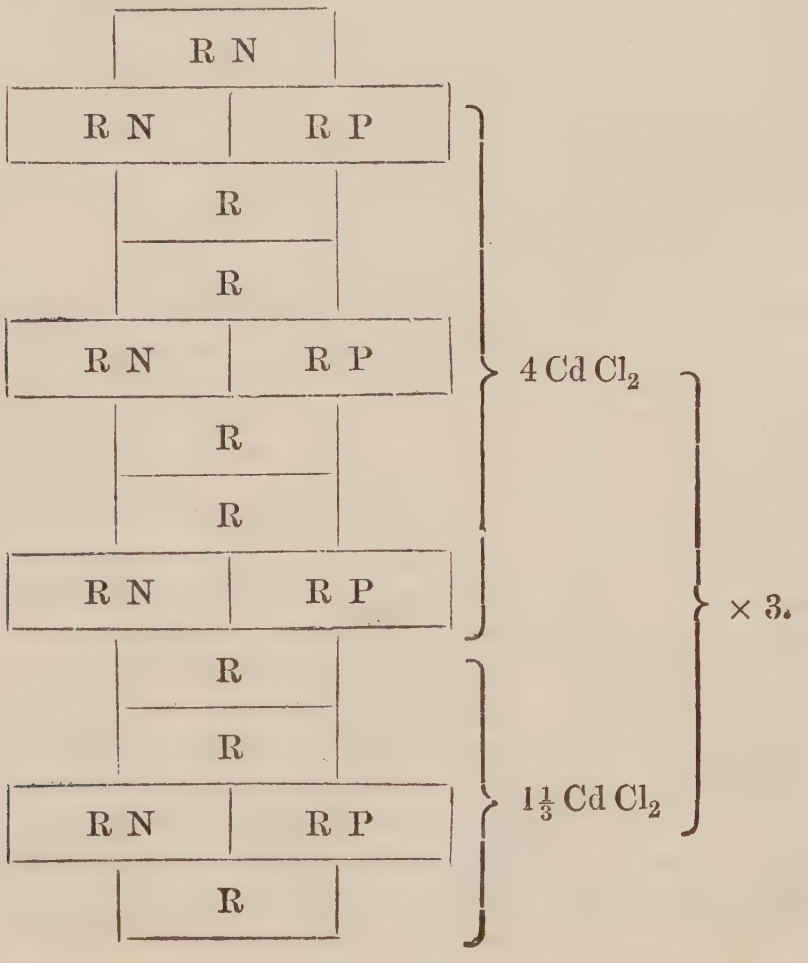
Summary of Results :—

			÷ by At. Wgts.	÷ by P = 1.	P = 4.
C	47·88		3·99	38·73	
H	7·71		7·71	74·8	
N	1·81	75·14	0·129	1·25	5·00
P	3·20		0·103	1·	4
O	14·54		0·908	8·81	
Cl	9·61	24·86	0·270	2·62	10·48
Cd	15·25		0·136	1·32	5·28
	100·00				

We thus find that Cd : Cl = 1 : 2, but the Cd Cl_2, as compared to the organic molecule, is about one third (of its theoretical quantity) in excess of its required quantity. Deducting the Cd Cl_2, and calculating organic molecule we obtain :—

		÷ by At. Wgts.	÷ by P = 1.	P = 4.
C	63·72	5·315	38·79	—
H	10·26	10·26	74·89	—
N	2·40	0·1714	1·25	5
P	4·25	0·137	1·	4
O	19·37	1·21	8·81	—
	100·00			

If we assume that of every four molecules one contains an amidated radical besides the ammonium radical, we obtain the following scheme :—



This salt was extracted with benzole after analysis, in which it swelled, but did not dissolve; some coloured matter was extracted. It is therefore different from the principal ox $Cd\,Cl_2$ salt, which was repeatedly dissolved in benzole and precipitated by alcohol, but is nevertheless very similar in composition.

Ox, $Cd\,Cl_2$ salt. $C_{39}\,H_{75}\,N\,P\,O_9 + Cd\,Cl_2$.
Man, $Cd\,Cl_2$ salt. $C_{39}\,H_{75}\,N\,P\,O_9 + Cd\,Cl_2 + \frac{1}{3}\,Cd\,Cl_2$.

There remain to be inquired into, and to be analysed if sufficient material can be collected:

(*a.*) The $Cd\,Cl_2$ salt extracted by ether.
(*b.*) The $Cd\,Cl_2$ salt extracted by alcohol at 45° C.
(*c.*) The body extracted by benzole.
(*d.*) The cause of the insolubility in benzole, and whether it is the excess of $Cd\,Cl_2$ or not.
(*e.*) The organic body combined with the $Cd\,Cl_2$ after removal of this salt.
(*f.*) The chemolytic products of the organic body.

Myeline Cadmic Chloride, from Ox White and Buttery Matter, and Cerebrine.—This myeline was a mixture of various specimens, similarly prepared. White matter, extracted with cold alcohol and ether; also buttery in the same manner; further, cerebrine deposited by ether extracts of white matter, were found to contain much phosphorus. The whole of the matters were therefore dissolved in hot absolute alcohol, and the solution allowed to cool. The filtrate was a saturated solution of myeline. This was allowed to stand, and deposited some gelatinous kerasine, which was filtered off. To the colourless clear solution $Cd\,Cl_2$ was added as long as a precipitate was produced. The white deposit was washed and redissolved in boiling absolute alcohol. Most of it dissolved, and was deposited on cooling after filtration on hot funnel. Some remained insoluble, and is here not any further considered. Some remained dissolved in the absolute alcohol after cooling, and was obtained after distillation of the alcohol. Some remained dissolved in the very last mother liquor. The following salt is the one deposited on cooling of the alcohol solution: white masses, little waxy, easily powdered, dried in vacuo.

Insoluble part.

Analyses.—(1.) Determination of phosphorus. 2·1008 grm. were fused with caustic soda, nitre, and carbonate; the residue was dissolved in H Cl, and the Cd removed by hydrothion. This yielded only 0·2272 Cd O, equal to 9·463% Cd, so that the loss surmised during the experiment was verified. To the acid filtrate $N\,H_4\,H\,O$ was added to alkalinity, whereby a precipitate was produced, proved subsequently to consist of calcium phosphate, with a trace of iron; this weighed 0·0178 grm., and its phosphorus was calculated as that of $Ca_3\,P_2\,O_8$ = 0·00342% P. To the filtrate magnesia mixture was now added, and the process yielded 0·2418 grm. $Mg_2\,P_2\,O_7$, equal to 3·214% P, which with the P in the lime salt, is 3·2174% P nett.

Nitric acid process.

(2.) Cd Cl and P determination by HNO_3 method. To avoid the loss of Cd, 2·0238 grm. of the salt were boiled with excess of nitric acid and silver nitrate 1 grm., in a long-necked assay flask, until destroyed. At one time the solution was bluish, and ultimately colourless; the Ag Cl deposited, and a little oil floated on top, which became solid on cooling. This remained with the Ag Cl, and caused some difficulty in the ignition of the latter, which required treatment with $H\,N\,O_3$ and H Cl, after which the Ag Cl behaved normally. It weighed

0·5533 grm., equal to 6·754°/₀ Cl. The Cd S next precipitated, and transformed into oxyde, with the precaution of treating the paper filter with HNO_3 previous to ignition, gave 0·2928 Cd O = 12·60°/₀ Cd. This latter figure requires 7·99°/₀ Cl, whereas the 6·754 °/₀ Cl found require only 10·65/ Cd. We have therefore here the Cd undoubtedly slightly in excess of the Cl, or, in other words, a loss of chlorine. The acid solution was now treated with excess of NH_4HO, and gave the same precipitate as analysis 1. It was not filtered off. The phosphoric acid was now precipitated by magnesia mixture, and yielded 0·1048 grm. $Mg_2P_2O_7$, equal to 1·466 °/₀ P. (See notes below.) Cl. Cd. P.

(3.) 0·4312 grm. gave 0·808 grm. CO_2 = 51·104°/₀ C, and 0·3466 grm. H_2O = 8·931°/₀ H. C. H

(4.) 0·5460 grm. dried at 80° C. during four hours, gave 12 c.c. gas at 20° C, and 767 m.m. B. K H O column = 18 m.m. Hg = 10·77 c.c. normal, or 2·46°/₀ N. N.

(5). Determination of Cl and P by fusion method. 1·432 grm. gave 0·4850 grm. Ag Cl equal to 8·37 °/₀ Cl and 0·1720 grm. $Mg_2P_2O_7$ equal to 3·354 °/₀ P.

Note on Analytical Methods.—There being a great deficiency of P in analysis 2), the Ag Cl was searched for this element. It was dissolved in ammonia and nitric acid alternately applied, and the Ag Cl ultimately filtered from the acid solution. The latter showed by molybdate test unmistakeable evidence of the presence of P. It is therefore probable that the fatty body which remained and was burned with the Ag Cl contained P. Further, the ultimate mother liquor of the magnesia precipitate was evaporated to dryness and fused with nitre. The solution in water with the molybdate test showed the presence of a large amount of P. *It is therefore clear that by this treatment with nitric acid, phosphorus in two forms may escape detection, and that the process is unsuitable for the determination of this element.* The uncertain and varying results of Couerbe and Köhler receive by this fact a partial explanation. On the other hand, it is proved that the method of fusion with nitre and sodium carbonate after previous digestion with caustic soda leye gives the whole of the chlorine and phosphorus contained in the salt, but is liable to engender a loss of cadmium. This metal seems however to be completely isolated by the nitric acid process, and I have therefore come to the resolution to determine Cd by the nitric acid method in one experiment, in which Cl and P are left out of consideration, and to determine P and Cl by the fusion method in another experiment in which Cd is left out of consideration.

Summary of Analyses :—

	Per cents.			
C	51·104			
H	8·931			
N	2·460			
P	3·285	mean of	3·217, 3·354	79·030
O	13·250			
Cl	8·370			20·970
Cd	12·600			
	100·000			100·000

Computation of Organic Molecule :—

	Per cents.	÷ by at ws.	÷ by P=1.	P=2.	P=3.
C	64·66	5·38	40·2		
H	11·30	11·30	84·4		
N	3·11	0·2221	1·65	3·30	4·95
P	4·15	0·1338	1·00	2·	3·
O	16·78	1·05	7·84		
	100·00				

Again this body is not a simple, but a compound salt, containing perhaps three molecules of myeline, in two of which an amidated radical is contained. The simplest formula that can be constructed is probably—

$$\left.\begin{array}{l} C_{40}\,H_{84}\,N_2\,P\,O_8\,Cd\,Cl_2 \\ C_{40}\,H_{83}\,N\;\;P\,O_8\,Cd\,Cl_2 \\ C_{40}\,H_{84}\,N_2\,P\,O_8\,Cd\,Cl_2 \end{array}\right\}$$

Paramyeline Cadmic-Chloride, $C_{38}\,H_{75}\,N\,P\,O_9$, $Cd\,Cl_2$, and its hydrate, $C_{38}\,H_{77}\,N\,P\,O_{10}$, $Cd\,Cl_2$, *from ox buttery after kephaloidine.* When the buttery matter dissolved in ether had been precipitated by alcohol and the kephaloidine been removed, the mother liquor on standing deposited some secondary kephaloidine and cholesterine. These were filtered off, the liquid precipitated with $Cd\,Cl_2$; the precipitate was washed with alcohol and pressed; it was next extracted with ether (which dissolved a small quantity of kephaloidine $Cd\,Cl_2$) until pure, dried, and analysed. The result of the analyses showed, that the body was a $Cd\,Cl_2$ compound, but there was no easy rationality between the elements of the organic molecule. The latter amounts to 77·49 °/₀ the $Cd\,Cl_2$ to 22·51 °/₀.

Treatment with Benzole.—It was found that the compound was entirely soluble in boiling benzole, and deposited a portion on cooling which was white and voluminous. Another portion remained dissolved in the cold benzole. The deposit was isolated by filtration, dissolved once more in boiling benzole, and was deposited as a swelled gelatinous mass; this was drained from benzole by blotting paper and dried. (Preparation 1235). The benzole solution which deposited nothing on standing in the cold was concentrated by distillation, and then precipitated by absolute alcohol, the precipitate washed and dried. (Preparation 1236.)

(*a.*) *Paramyeline Cadmic-Chloride.* — $C_{38}\,H_{75}\,N\,P\,O_9$, $Cd\,Cl_2$, insoluble in cold benzole.

Analyses.—The analyses were carried out in the usual manner, but the Cd was disregarded, as the fusion method is not reliable with this element.

(1.) 1·1505 grm. dried at 80° gave,—

(*a.*) 0·384 grm. $Ag\,Cl$ = 8·255 °/₀ Cl, [to this a quantity of 13·02 °/₀ Cd. would correspond.]

(*b.*) 0·1400 grm. $Mg_2\,P_2\,O_7$, equal to 3·396 °/₀ P.

(2.) 0·441 grm. gave 0·7970 grm. CO_2 = 49·288 °/₀ C and 0·3294 grm. H_2O = 8·299 °/₀ H.

(3.) 0·5321 grm. gave 7·7 c.c. gas at 22° C and 765 m.m. B.; KHO column = 16·4 m.m. Hg, gas = 6·8 c.c. normal = 1·598 °/₀ N.

Summary :—

Hypothetical	C	49·288	78·725
	H	8·299	
	N	1·598	
	P	3·396	
	O	(16·144)	
	Cd	(13·020)	21·275
	Cl	8·255	
		100·000	100·000

Computation of Organic Molecule :—

	Per cent.	÷by At wts.	÷by P = 1.
C	62·607	5·217	37·5
H	10·541	10·541	75·8
N	2·029	0·144	1·0
P	4·313	0·139	1·0
O	20·510	1·281	9·2
	100·000		

The formula $C_{38}H_{75}NPO_9$ gives an atomic weight of 720, but the atomic weight calculated from the $CdCl_2$ is only 677. There is therefore still an irrationality between the chloride and the organic molecule, in the sense of the metallic salt being in excess.

Paramyeline Hydrate Cadmic-Chloride.—$C_{38}H_{77}NPO_{10}$, $CdCl_2$. (C_{37} ?) (Prep. 1236). Soluble in cold benzole.

Analyses.—All preparations dried at 80°.

(1.) 0·5000 grm. gave 0·8310 grm. CO_2 = 45·237°/₀ C; and 0·3570 grm. H_2O = 7·933°/₀ H.

(2.) 0·420 grm. gave 6·2 c.c. gas at 26 c. and 764 m.m. B.; KHO = 16 m.m. Hg; equal to 5·38 c.c. gas normal = 1·602°/₀ N.

(3.) 0·9380 grm. gave 0·3660 grm. AgCl = 9·652°/₀ Cl (to this quantity would correspond 15·222°/₀ Cd); further, 0·112 $Mg_2P_2O_7$ = 3·334°/₀ P.

Summary :—

	C	45·327	75·126
	H	7·933	
	N	1·602	
	P	3·334	
	O	(16·930)	
	Cd	(15·222)	24·874
	Cl	9·652	
		100·000	100·000

Computation of Organic Molecule :—

	Per cents.	÷ by At. Wgts.	÷ by P = 1.
C	60·334	5·028	$C_{36·9}$
H	10·559	10·559	$H_{77·6}$
N	2·132	0·152	$N_{1·1}$
P	4·437	0·136	P_1
O	22·538	1·408	$O_{10·3}$
	100·000		

Nearest formula $C_{37} H_{77} N P O_{10}$, more probably $C_{38} H_{77} N P O_{10}$, with atomic weight 738 ; the $Cd Cl_2$ is greatly in excess of the molecule, and leads only to 552 atomic weight.

Diagnosis.

The solubility of this paramyeline in ether distinguishes it from myeline, which is only little soluble in ether ; its solubility in ether alcohol separates it from the kephaline group, from which it also differs by the insolubility of its cadmium compound in ether ; it thus approximates by its solubilities most to the group of the lecithines, to which it also shows analogies by its bearing during chemolysis. But it differs from the lecithines *by its platinic chloride salt being insoluble in ether*, as was ascertained by the following experiment :—

Attempt to transform the foregoing Cd Cl_2 salt into Pt Cl_4 salt.—A quantity of 10 grm. of the analysed bodies was triturated very finely, first by itself, and then with H Cl, diluted with an equal volume of water, and then allowed to stand for about three hours. It was next thrown on a filter and washed with water ; when the excess of H Cl and Cd Cl_2 had been washed away, the substance became mucilaginous and filtration ceased. The liquid in the funnel was poured off, and the soft yellowish white matter treated with cold absolute alcohol. This left a flaky white matter undissolved, which, however, dissolved in hot absolute alcohol, and was deposited on cooling in minute white granules, the alcohol remaining saturated with it, so as to give a precipitate with Pt Cl_4, and leave a matter on evaporation. The first cold alcohol solution was precipitated completely with Pt Cl_4, and the yellow precipitate isolated, treated with ether, *which extracted nothing*, and dried in vacuo. It weighed only 2·13 grm., and on analysis was found to contain 80·45% of organic matter, and 19·55% of Pt Cl_6, so that it was evidently formed upon the type of the platino-chlorides-hydrochlorates : viz., 2 paramyeline + 2 H Cl + Pt Cl_4, with a slight excess of H Cl over that required by Pt Cl_6, and also a slight excess of organic matter, so common in the compounds of the kephaline series, and explained by the chemolytic action of water. On the whole, this method of transformation is unsatisfactory, and does not deserve to be repeated, particularly on account of the small yield.

Cannot be washed with water.

Chemolysis of the foregoing salts by $Ba H_2 O_2$. 10 grm. were boiled with concentrated caustic baryta, containing excess of crystals, for one hour. Solution and precipitate were little coloured ; they were separated by filtration.

The precipitate was washed and decomposed with cold H Cl. The insoluble part was washed to near freedom from H Cl, and then dissolved in hot absolute alcohol. Immediately on cooling a *first crystalline deposit* formed, which was filtered off. The solution evaporated to one half deposited, over night, white zeolithic masses of *light crystals*, being a *fatty acid*. The solution further evaporated deposited a *crystalline coloured fat* fluid while hot, nearly solid on cooling. These products will be described lower down as *first*, *second*, and *third acids*.

First crystalline deposit.

Second and third deposit.

The barytic solution was treated with excess of CO_2, and heat, filtered, concentrated, and precipitated with Pb acetate. The precipitated *Pb glycerophosphate* was removed; the excess of lead precipitated by H_2S, the solution filtered, evaporated to dryness, treated with a few drops of HCl to expel acetic acid; the residue dissolved in water, and filtered from a small quantity of *green base;* mixed with $PtCl_4$ and absolute alcohol, which precipitated *choline* and *neurine Pt* Cl_4, mixed with some $BaCl_2$. The Ba was cautiously removed by H_2SO_4, and the crystals obtained pure.

The solution. Pb glycero-phosphate. Pt Cl₄ salts.

First Crystalline Deposit.—This having been deposited immediately on cooling, was filtered off, washed with cold absolute alcohol, pressed between paper, and represented white microscopic crystalline needles, much resembling the needles obtained from cerebrine by the action of HCl or H_2SO_4. They soften at 75°, at 84° bubble and blister from loss of fluid (either water or alcohol), and then set dry. The mass does not fuse at 95°, at 105° maintains its shape, but is soft like wax when kneaded between the fingers. At 120° it is transparent, and nearly fused. At 127° it is a colourless, transparent oil, but very viscid, and not mobile. On cooling it becomes hard, and remains translucent, like milky glass or porcelain, and is not crystalline, but brittle. This body has not yet been any further examined. The crystals, pressed between paper and dried in air, when boiled with absolute alcohol, dissolve in great part, but leave a *fused* or *stearoconotised* portion undissolved behind; this adheres to the glass, and remains insoluble on boiling with much alcohol; after removal of the alcohol it cracks, but remains adherent to the glass. The alcoholic solution gives copious white precipitate with Pb acetate. Myeline behaves in a similar manner. On the other hand none of the known fatty acids observe such conduct, and they are therefore excluded from consideration.

The Second Deposit, or Crystals of Fatty Acid.—This crystallised from the absolute alcohol solution after concentration to one half on standing over night. It was very different from the first, being in dense balls of zeolithic needles, and breaking up on pressure, much like margaric acid so called. It was filtered off, washed with some absolute alcohol, and pressed between bibulous paper, dried in air. White; softens at 49°, fuses at 50°, entirely liquid at 54°. Solidifies crystalline at 50° to 51°, thermometer immersed. (These fusing points correspond to myristic acid, containing a little lauric, but the analyses to be related do not confirm this hypothesis.)

Second deposit.

The acid was dissolved in a small quantity of warm absolute alcohol, and precipitated by baric acetate solution; the precipitate was filtered, and washed three times with absolute alcohol, and when dry weighed 0·5272 grm.

Analyses.—(1.) 0·1166 grm., dried at 70° C., and ignited with H_2SO_4 and HNO_3 alternately, until white gave 0·0404 grm. $BaSO_4$ = 20·37% Ba.

(2.) 0·2053 grm., dried at 80°, burned with chromate, gave 0·4294 grm. CO_2 = 57·042% C, and 0·1824 grm. H_2O = 9·871% H.

Summary of Analyses:—

	%	÷ by At. Wgts.	÷ by Ba = 1.
C	57·042	4·753	31·98
H	9·871	9·871	66·42
O	12·72	0·795	5·35
Ba	20·37	0·1486	1·00
	100·000		

leading to 2 ($C_{16} H_{31} O_2$) Ba, $H_2 O$, therefore a salt very nearly isomeric with palmitate or margarate. These few tests exhausted the material.

Third deposit. Third acids.

The Third Deposit.—The solution filtered from second deposit was evaporated until an oil collected on its top, and allowed to cool. The oil became semisolid. The fatty matter was filtered off, washed with water, and dissolved in watery ammonia. It formed a turbid, slimy solution, which, after heating with more water, could be filtered. To the solution Pb acetate was added, the curdy precipitate filtered off, washed with water, and dried over $H_2 S O_4$. It was next extracted with ether, and residue dried and weighed = 2·57 grm. The ethereal yellow extract was distilled to dryness from a previously weighed flask, and found = 2·7175 grm. The exact study of these products requires much larger quantities of material than were at my disposal.

Glycero-phosphate of Pb.

The Glycerophosphate of Lead was filtered and washed, and boiled twice with large quantities of water, and washed on a new filter with hot water. The white salt was dried over $H_2 S O_4$; it shrank very much to a horny coloured mass, and weighed when dry 1·5 grm. Of this 1·409 grm. were burned, and left 1·097 grm. $Pb_2 P_2 O_7$; it was consequently pure glycerophosphate, which should leave 1·098 grm. pyrophosphate.

Basic body.

The Green Basic Body.—When all acid was driven off from the choline-neurine hydrochlorate, after removal of Pt by $H_2 S$, and the residue dissolved in water, a greenish body remained undissolved, which from the mode in which it was obtained may be considered as the hydrochlorate of a base. Insoluble in water, it dissolved easily in absolute alcohol, forming a dark red solution. In this $Pt Cl_4$ produced neither precipitate nor change. (The solution was lost, but it is known from other experiments that the $Pt Cl_4$ compound is precipitated by ether from its alcohol solution.)

Choline and neurine salt.

The Choline and *Neurine Pt Cl_4* was filtered, and washed with absolute alcohol, dried in air, and redissolved in a minimum of water, filtered from a slight yellowish, grey, insoluble deposit. As it did yet contain $Ba Cl_2$, it was again transformed into simple chloride by removal of the Pt by $H_2 S$, and evaporation. The residue was extracted with absolute alcohol in small quantities, as long as the extracts gave any precipitate with $Pt Cl_4$. $Ba Cl_2$ in white crystals was left behind. To the united extracts $Pt Cl_4$ was added as long as any precipitate ensued, excess of $Pt Cl_4$ being avoided. The precipitate, yellow and powdery at first, became orange brownish during drying, and assumed a more dense and crystalline state. It weighed 1·6 grm. air-dry, equal to 0·532 grm. choline and neurine. This corresponds to only 3·35 grm. of the body taken for chemolysis, and even if the larger formulæ were to prevail for this compound, the choline-neurine obtained would not represent half the nitrogen contained in the original $Cd Cl_2$ salt.

Computation of the Quantities of Products obtained in this Chemolysis.

$Cd Cl_2$ to be deducted	2·251 grm.
First crystals from alcohol (estimated)	1·000 ,,
Second crystals, fatty acid (Ba salt = 0·5272 grm.)	0·400 ,,
Assumed oleic acid (from 2·7175 grm.) of Pb salt	1·993 ,,
Assumed margaric acid (from 2·57 grm.) of Pb salt	1·834 ,,
Glycerophosphoric acid (from 1·5 grm. Pb salt	0·684 ,,
Choline and neurine (from 1·6 grm. $Pt Cl_4$ salt)	0·532 ,,
Loss in operations, green base, &c.	1·306 ,,
Total	10·000 ,,

Myeline-Cadmic-Chloride. — $C_{84} H_{166} N_3 P_2 O_{18} + 2 Cd Cl_2$ from ox, white matter. This preparation constitutes the largest bulk of the compounds of the myeline class obtained in this research. It contains the myeline which is the least soluble, as is shown by the mode in which it was obtained. The white matter, from which cold absolute alcohol had extracted the more soluble compounds :—from which ether had extracted the kephaline and cholesterine, and which then appeared to consist mainly of bodies of the cerebrine series, was found to contain much phosphorus. It was finely powdered and boiled with absolute alcohol, the solution was decanted from the stearoconote, and some kephaline, filtered hot, and allowed to stand. Three successive deposits were separated, which will be described under the record relating to the cerebrine, phrenosine, and kerasine series. The alcoholic filtrate on standing several days in stoppered bottles deposited some kerasine in the form of gelatinous granules and membranes consisting of microscopic waves of needles. The alcoholic filtrate from this was saturated with a body, which gave a copious white precipitate with $Cd Cl_2$. It was necessary to combine the whole with this salt, in order to remove cholesterine, of which there was also much contained in the fluid, as shown by ultimate concentration of the mother liquor. The copious precipitate was washed with absolute alcohol, then suspended in and exhausted with ether, and dried, first in the open air, then under the air pump, with the aid of sulphuric acid. This process of drying lasted many weeks, as the alcohol and ether clung pertinaceously to the substance. It had to be powdered many times, and the sulphuric acid used for drying had to be frequently renewed before the substance appeared dry. The half dried substance when heated in a water bath to 90° C. became a transparent, viscous, soft mass ; on cooling this became again hard and pulverizable, it was thus powdered and again exposed to 80° C., when it again became soft, but less so than at first ; cooled and ground in mortar a second time it now retained its pulverulent nature on heating and did not fuse or become soft. From this it is probable that water, and alcohol, and ether were strongly combined with this body, and that the observed fusion was really a kind of solution in a small portion of these agents. The portions used for the following analyses were then dried, and subsequently at 70° C. till their weight was constant.

Large preparation.

How obtained.

Mode of drying.

Analyses.—(1) 1·0233 grm. fused as above described gave 0·1230 $Cd O = 10·983$ °/₀ Cd ; 0·3050 $Ag Cl = 7·373$ °/₀ Cl ; and 0·118 $Mg_2 P_2 O_7 = 3·220$ °/₀ P.

(The 10·983 °/₀ Cd require 6·961 °/₀ Cl. The 7·373 °/₀ Cl require 11·630 °/₀ Cd, so that the Cd is deficient to the extent of 0·647 °/₀, the result probably of volatisation during fusion.)

(2.) 0·4466 grm. burned with chromate gave 0·8570 grm. $C O_2 = 52·234$ °/₀ C, and 0·346 grm. $H_2 O = 8·607$ °/₀ H.

(3.) 0·5424 grm. gave 11c.c. gas at 19° C. and 753 m.m. B. ; KHO column = 17 m.m. Hg, equal to 9·74c.c. gas normal = 2·246 °/₀ N.

Summary of results :—

C	52·334	81·644	
H	8·607		
N	2·246		
P	3·220		
O	15·237		
Cd	10·983	18·356	
Cl	7·373		
	100·000	100·000	

App. No. 5.

On the Chemical Constitution of the Brain, by Dr. Thudichum.

Computation of free Molecule :—

	Per cents.	÷by At. Wts.	÷by P=1
C	64·100	5·341	42·05
H	10·542	10·542	83·00
N	2·750	0·196	1·54
P	3·943	0·127	1·00
O	18·665	1·166	9·18
	100·000		

Theory of Atoms :—

42	C	504	783	
83	H	83		
$1\frac{1}{2}$	N	21		
1	P	31		
9	O	144		
1	Cd	112	183	
2	Cl	71		
		966	966	

Of course the theory with P = 1 is inadmissible on account of the N, which compels us to double the empirical formula to $C_{84}\,H_{166}\,N_3\,P_2\,O_{18}$, $+2\ Cd\,Cl_2$. Taking Cl as found, and Cd equivalent to it (allowing a slight loss) leads to 963 as at. w. of compound, which is almost identical with theory from free body. Consequently, we have :—

Cl : P = 2 : 1 or better = 4 : 2
Cl : N = 2 : $1\frac{1}{2}$ „ „ = 4 : 3

It is therefore possible or necessary to assume that we have here a mixture or compound in atomic proportions of two bodies, one of which contains only one atom of N, while the other contains two, thus :—

$$\left.\begin{matrix} C_{42}\,H_{83}\,N\,PO_9 + Cd\,Cl_2 \\ C_{42}\,H_{82}\,N_2PO_9 + Cd\,Cl_2 \end{matrix}\right\} = C_{84}\,H_{165}\,N_3\,P_2\,O_{18} + 2\ Cd\,Cl_2$$

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c. Sub-group of Lecithines.

Explanation: Lecithine virtually found, not actually isolated.

In the chapter containing the description of the method pursued for the isolation of immediate principles, I have repeatedly stated that lecithine had been found, but have accompanied the statement by a query, as indicating that there was yet some doubt on the subject. This may be at once explained by the statement that the lecithine, when isolated in the only form in which it can at present be separated from the other brain matters, namely, as platinic chloride hydrochlorate salt, immediately after isolation began to decompose, and completed this decomposition during the steps necessary for its purification. The yellow salt, precipitated from ether by alcohol, when placed in the vacuum over sulphuric acid, became covered with oily drops (oleic acid), and the remaining yellow salt entirely lost its solubility in ether. On analysis of this insoluble part it was found to correspond to the probable composition of a plati-

Lecithine, how and when decomposed.

Pt salt lecithine minus oleyl.

num salt of a body having the composition of lecithine minus an atom of oleyl. But slight irregularities made it probable that a small part decomposes further, and leaves a platinum salt, free from fatty acid radicles, yet containing the radicles of glycerophosphoric acid and choline in combination. These circumstances produced conditions of such complication, that the time and opportunity was not sufficient for disentanglement, and the presence of lecithine in the brain is therefore not considered as proved, but only as probable.

Decomposition observed by others.

The decomposibility of lecithine from eggs has already been pointed out by Strecker; the ether solution of the platinic chloride salt he found to deposit crystals of choline platinic chloride on standing. This decomposibility was also no doubt the main cause of Gobley's non-success in his attempts at its isolation. I find that it decomposes rapidly on standing in absolute alcohol by itself; the liberated margaric and oleic acids in the nascent state form ethylic ethers, which settle as a heavy oil to the bottom of the vessel containing the solution. This oil then forms a material of almost inextricable confusion and complication, and has no doubt been the main cause of the frequent failures of attempts at brain analysis. When freed from all matters which can be precipitated by platinic chloride, cadmic chloride and lead acetate, applied in succession with all precautions, and when liberated from all alcohol it is a viscid oil, which partly crystallises at low temperatures, and at lower ones congeals entirely. When heated it does not observe the bearing of either the phosphorised bodies, or fats or fatty acids, but boils at a temperature near that of boiling mercury, and distils freely above 360° C., yielding an oily distillate in which crystals form on standing. This distillate is the pyrokephole of the alphabetical list; the original oil, unpurified, is the main ingredient of Couerbe's éléncephol, of the last oily of this research. During the early operations of brain-extraction the decomposition of lecithine is but slight, and the formation of the ethers insignificant. It begins to become more copious after concentration of the absolute alcohol extracts from white and buttery matter, by distillation of the absolute alcohol. It is accelerated by the treatment with platinic chloride and hydrochloric acid, but a large quantity of soluble in ether platinum salt is nevertheless obtained. The final decomposition is accelerated by the necessary separation of kephaline, which involves treatment with hydrothion in ether solution, and precipitation with absolute alcohol, when kephaline remains with the platinic sulphide, while lecithine as hydrochlorate with much hydrochloric acid remains in the alcohol solution. The hydrochloric acid has now to be removed by silver oxyde, the excess of the latter by hydrothion; the excess of hydrothion by carbonic acid and slight warming. In this solution platinic chloride produces a good precipitate of lecithine salt, of which most is yet soluble in ether. But after precipitation by alcohol and placing in the drier, the long course of severe chemical influences is, as it were, completed, and the shaken constitution of the lecithine succumbs to the influence of time and liberated hydrochloric acid.

Oily bodies formed ethylic ether of fatty acids.

Distillate = pyrokephole.

Progress of decomposition of lecithine during manipulation.

We may therefore say that lecithine has virtually, though not actually, been isolated from the brain. It has yielded all the products of decomposition described by Strecker, and also the compounds, and has observed the bearing of a compound in a state of tension, or easy decomposibility. I have no doubt that means will be found for its isolation in its integrity. Perhaps the above process followed by me, if carried out with the greatest despatch by working it during night and day without intermission, would lead to the desired result. For the decomposition no doubt requires time as an essential element. But other processes are perhaps preferable. Thus if all phosphorised matters were precipitated from the first extract

Processes of isolation of lecithine to be adopted in the future.

of white matter, or buttery matter by cadmic chloride; the precipitate freed from kephaline compound by ether; the remaining mixture of myeline and lecithine cadmic chloride salt freed from cadmic chloride by water and dialysis; and the mixture of myeline and lecithine separated first by a little ether, which would leave most myeline insoluble, but dissolve all lecithine; if now to this solution ethereal solution of platinic chloride were added, all myeline would fall, and all lecithine remain in solution. This could now be precipitated by alcohol, and the precipitate analysed; or the precipitate could be freed from platinum and hydrochloric acid as above described, and the solution evaporated. The compound of lecithine with lead is very soluble in alcohol, even of inferior strength, while the compound of myeline with lead is quite insoluble even in boiling alcohol. Perhaps this different behaviour may be used for their separation after they are isolated from other phosphorised matters and cholesterine by the cadmic chloride and ether treatment.

B.—Group of Nitrogenised Principles.

(*a.*) *Sub-Group of the Cerebrines.*

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General Peculiarities of the Group.

In the classification I have given cerebrine and its several varieties as the first term of the group of principles containing four elements, C, H, N, and O, termed the nitrogenised group of immediate principles of the brain. I have shown in the historical appendix that the term cerebrine was first used by Kühn, and later by Lassaigne, for ill-defined products of their operations; later by Gobley to signify what was no doubt mainly a mixture of bodies belonging to this group, with some myeline, but the representative body of this group, the cerebrine $C_{17}\,H_{33}\,N\,O_3$ (or $C_{34}\,H_{66}\,N_2\,O_6$) was first discovered by Müller, and well characterised by its freedom from phosphorus, and the relatively large amount of nitrogen which enters into its composition. By the present research it is now found to be apparently the lowest representative of a group of immediate principles of the brain which are free from phosphorus, contain nitrogen, and vary in the number of carbon atoms, which they contain for each nitrogen atom between 17 and 48. Whatever may be the ultimate explanation of these differences of composition must be left for future inquiry. Meanwhile it is certain that these differences do but slightly affect the external appearance and bearing towards solvents of these

bodies, so that by describing the general properties of one we describe the general properties of all members of the group, while differentiating characters and means are most difficult of discovery and application.

They are all white, like snow, but are capable of becoming in part transparent, like wax. They are deposited from hot alcoholic solutions in minute balls of still more minute, *i.e.*, microscopic particles, which may be termed crystalline, but have no claim to be termed crystallised. [The balls of myeline are very similar in appearance, but their elements are much more distinctly crystallised than those of the cerebrines.] General characters of cerebrines.

The cerebrines are all soluble in hot alcohol, particularly absolute alcohol, and deposited on cooling; they are very little soluble in cold absolute alcohol, much less soluble indeed than myeline, which can thus be separated from the cerebrines. The mixture is dissolved in hot alcohol and allowed to cool; nearly all cerebrine falls down, much myeline remains in solution. The deposit is separated from the liquid and subjected to this treatment until it is free from phosphorus. Soluble in alcohol.

Solubility in Water.—The cerebrines are almost insoluble in water, though they swell with it, particularly on boiling. One grm. of purified cerebrine (from ox, third portion of Nr 2 of my preparation list) was powdered and boiled in 100 c.c. of water, filtered through force filter, and 50 c.c. of the filtrate were evaporated on the water bath to dryness in a platinum dish, which increased in weight by 0·025 grm. Consequently one part of this cerebrine was soluble in 2,000 parts of water. Solubility in water.

The cerebrines are quite insoluble in cold benzole. Thus a quantity (from ox, Nr 3) was shaken with benzole; it became swelled and quite transparent, so as to almost disappear from sight. The benzole was filtered off and evaporated, and did not leave a vestige of matter behind. But in hot benzole the cerebrines are extremely soluble, and on cooling are deposited as a gelatinous mass, which requires agitation before it can be filtered. From the hot solution cold alcohol precipitates white flakes. This treatment facilitates the separation of the cerebrine from the benzole. Bearing with benzole.

The cerebrines are almost insoluble in either cold or hot ether, and are by this solvent easily purified from kephaline and its relatives, from much myeline, and from lecithine, and also from cholesterine and fats. But the separation from myeline cannot be effected so easily by ether as by the absolute alcohol treatment above described. Bearing with ether.

In order to ascertain the bearing of cerebrine under different circumstances with hydrothion, three experiments were made with cerebrine from man, Nos. 4 and 5. A quantity was suspended in ether and H_2S passed through the mixture. No change took place. A mixture of cerebrine with alcohol and some HCl also remained unaltered. A mixture of cerebrine with ether and alcoholic $CdCl_2$ formed a yellow precipitate. Cerebrine therefore behaves neutrally towards H_2S, unlike kephaline, which seems to combine with it, and then retains metallic sulphides in solution in ether. Bearing with H^2S.

Cerebrine and Phrenosine from Brain of Man.

Cerebrine (No. I.) $C_{17}H_{33}NO_4$ *from Brain of Man, by Baryta Process.*—This experiment was performed on two human brains (typhus) after the precedent of Müller. The organs, hardened in alcohol, were transformed into a pulp and boiled with baryta water; the solid residue Cerebrine No. 1.

was filtered off, and after washing exhausted with boiling alcohol. The latter deposited *cerebrine*, which was extracted with ether, recrystallised from alcohol, and analysed. Dried in hot water oven at about 90° C, the powder became slightly caked, but did not become coloured or show signs of decomposition.

Analyses.—(1.) 0·5744 grm. burned with lead chromate gave 1·3843 grm. $C O_2 = 65·7\%$ C, and 0·5610 grm. $H_2 O = 10·8\%$ H.

(2.) 0·3609 grm. gave 13·9 c.c. of N at 11° C, and 760·69 B. potash column 240 m.m. = 12·7 c.c. N normal or 4·4% N.

Summary :—

		% found	÷ by N=1.
C	-	65·7	17·1
H	-	10·8	34·4
N	-	4·4	1·
O	-	19·1	3·8
		100·0	

leading to $C_{17} H_{33} N O_4$, or $C_{17} H_{33} N O_3 H_2 O$. This is the only time that I have obtained this cerebrine ; all other preparations, even by the same process, contained much less nitrogen, or assuming nitrogen as 1 as the term of comparison many more atoms of C, H, and O than this. It differs from Müller's cerebrine only by containing nearly an atom of $H_2 O$ more than this.

Man, No. II.

Phrenosine (No. II.) ($C_{35} H_{69} N O_8$).—This was also obtained by baryta process in exactly the same manner as No. 1, but was found to contain only half the amount of nitrogen, or it contained, in addition to $C_{17} H_{33} N O_4$ the complex $C_{18} H_{36} O_4$, which is not unlike Koehler's myelomargarine or Otto's cerebrine, both said to be free from N. But I have never found any substance of the cerebrine group to be free from nitrogen, and am rather inclined to assume that the true molecular formula of cerebrine No. 1 is $C_{34} H_{69} N_2 O_8$, and therefore may be considered as the diamido form of the radical of which phrenosine is the mono-amidated form.

Analyses.—(1.) 0·3188 grm. gave 0·7734 grm. $C O_2 = 66·1\%$ C, and 0·3180 grm. $H_2 O = 11·0\%$ H.

(2.) 0·3729 grm. gave 7·5 c.c. N at 15° 5 C.; H K O column 252 m.m. Bar. = 756·1 m.m. = 6·7 c.c. normal or 2·2% N.

		% found.	÷ by N = 1.
C	-	66·1	35·1
H	-	11·0	70·6
N	-	2·2	1·
O	-	20·7	8·1
		100·0	

leading to $C_{35} H_{69} N O_8$. I preserve this and all other cerebrines not produced from promiscuous materials for future study, particularly in the hope of meeting with preparations of similar empirical composition with which they might be united.

From the foregoing phrenosine ether extracted, previous to analysis, a

body which after distillation of the ether remained as a gelatinous cheesy cake, drying up to a thin film, probably myeline.

Cerebrine and Phrenosine (III.) ($C_{24}H_{47}NO_5$). This body was obtained without the intervention of baryta. The brain pulp was simply boiled with alcohol, the extract filtered, and allowed to cool; the deposit was filtered off. On attempting to redissolve this in boiling alcohol a portion fused together; the mass was therefore powdered again and extracted with much alcohol at 45°. The insoluble portion proved to be stearoconote (Couerbe), soluble in benzole and reprecipitated by alcohol. The cerebrine and phrenosine from the alcohol extracts, after extraction with ether, had composition :—

Cerebrine No. III.

Analyses.—Dried about 90° C. (1.) 0·3486 grm. gave 0·8565 grm. $CO_2 = 67·01\%$ C; and 0·3471 grm. $H_2O = 11·06\%$ H.

(2.) 0·4836 grm. yielded 13·3 c.c. of gas at 19° C. K H O = 238 m.m.; Bar. = 752· m.m., equal to 12·3 c.c. gas normal = 3·19% N.

		% found.	÷ by N=1.	÷ by N=4.
C	-	67·01	24·6	98·
H	-	11·06	48·7	195·
N	-	3·19	1·	4·
O	-	18·74	5·1	20·
		100·00		

If we multiply these figures by × 4, and then deduct two molecules of $C_{17}H_{33}NO_4$, or one molecule of $C_{34}H_{67}N_2O_8$, there remain about two molecules of $C_{34}H_{69}NO_8$. We can consider this body as being a mixture of :—

1 mol.	$C_{34}H_{67}N_2O_8$	cerebrine.
2 mols.	$C_{34}H_{69}NO_8$	phrenosine.
	$C_{34}H_{69}NO_8$	
	$C_{102}H_{205}N_4O_{24}$	(See cerebrine Nos. IV. and V.)
Found	$C_{98}H_{195}N_4O_{20}$	

Cerebrine and Phrenosine IV. and V. ($C_{25}H_{51}NO_6$.) This was extracted from the brains by alcohol at 45° C; it was next extracted by cold alcohol, then cold ether, recrystallised from alcohol, and washed with ether.

Man. cerebrine IV. and V.

Analyses (1.) 0·3914 grm. (dried below 90° C, at which temperature it became coloured), gave 0·9388 grm. $CO_2 = 65·41\%$ C, and 0·3862 grm. H_2O, equal to 10·96% H.

(2.) 0·3923 grm. yielded 10·8 c.c. gas at 19° C, and 752 m.m., which, corrected, was equal to 3·0% N.

	% found.	÷ by N = 1.	N = 4.
C	65·41	25·4	101·6
H	10·96	51·2	204·8
N	3·00	1·	4·
O	20·63	6·	24·
	100·00		

The hypothesis which I applied to Cerebrine III. fits the present preparation even much better.

Required - - C_{102} H_{205} N_4 O_{24}
Found - - $C_{101\cdot6}$ $H_{204\cdot3}$ N_4 O_{24}

These figures are in effect identical, and their harmony strongly supports the hypothesis.

Cerebrine and Phrenosine, VI., VII., VIII.

Cerebrine and Phrenosine VI., VII., and VIII. was prepared like the foregoing, and analysed.

(1.) 0·2837 grm. gave 0·6903 grm. CO_2 = 66·35 °/₀ C, and 0·2814 H_2O = 11·01 °/₀ H.

(2.) 0·4860 grm. gave 10·7 c.c. gas at 20° C, and 763 m.m. B, KHO 275 m.m. = 9·45 normal, or 2·43 °/₀ N.

	°/₀ found.	÷ by N = 1.	× 17 N.
C	66·35	31·9	542·3
H	11·01	63·6	1081·2
N	2·43	1·	17·
O	20·21	7·3	124·1
	100·00		

Applying to this body the foregoing hypothesis, I find that it may be considered as a mixture of 15 molecules of phrenosine, C_{34} H_{67} N O_8, with one molecule of cerebrine, C_{34} H_{67} N_2 O_8, which requires C_{544} H_{1072} N_{17} O_{128}, while $C_{542\cdot3}$ $H_{1081\cdot2}$ N_{17} $O_{124\cdot1}$ are found, figures which again show a surprising degree of harmony, considering the very large numbers of atoms involved in the calculation.

It is thus shown that different human brains yield immediate principles of the cerebrine series, which apparently greatly differ in elementary composition, though they are very similar in chemical reaction; but, by a simple hypothesis, they are reducible to two primary forms, mixed in varying proportions—

Mono-amidated form - C_{34} H_{67} N O_8 phrenosine
Diamidated form - C_{34} H_{68} N_2 O_8 cerebrine.

That the mono-amidated form really exists will be shown immediately; but there are reasons for assuming the existence of more complicated forms in mixtures, in which, besides the foregoing forms, bodies such as C_{51} H_{101} N O_{12}
C_{51} H_{100} N_2O_{12}
C_{51} H_{101} N_3O_{12}
may be engaged, or even an hypothesis dealing with these latter bodies, may in some instances take the place of the more simple hypothesis.

b. Sub-Group of Phrenosines.

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Ox cerebrine.

Recrystallisation and Separation by Lead Treatment of the Cerebrine Group of Immediate Principles from Ox-brain.—The white matter, completely exhausted with ether and cold alcohol, was repeatedly recrystallised from alcohol, of 85 °/₀, and the stearoconotes were separated. When the precipitate dissolved freely and entirely in new alcohol, the solution was treated with a hot alcoholic solution of lead acetate, and a little ammonia. [The precipitated lead compound, which I will term *lead cerebrinate*, was washed, decomposed in hot alcohol by H_2S, filtered hot from PbS. The solution, on cooling deposited *cerebrinic acid*, which was filtered off and washed. It was by recrystallisation obtained in three portions, which will be treated of on a future occasion.] The alcoholic solution from which the Pb precipitate had been removed, and which contained all matters not precipitated by lead under the circumstances, besides some Pb acetate and NH_3 acetate, on cooling, deposited white *phrenosine*, which was filtered off and washed. It was next recrystallised from alcohol of 83 °/₀, during which some secondary lead salt became insoluble, and was removed. The phrenosine was again dissolved in hot alcohol, and a trace of lead removed by H_2S. On cooling, phrenosine and a solution were obtained which were again separated by the filter. The phrenosine was recrystallised from absolute alcohol, and yielded, on cooling, *purest phrenosine, part* 1, which was dried. The mother liquor of this was distilled to one-sixth, and, on cooling, deposited *purest phrenosine, part* 2. The rest of the liquid evaporated left *purest phrenosine, part* 3.

Pb cerebrinate and cerebrinic acid.

Purest Phrenosine, Part 1.—This was dried for days over H_2SO_4 in air pump, and finally, the portions used for analysis were dried at 65° C.

(1.) 0·5218 grm. gave 1·2743 grm. CO_2 = 66·60 °/₀ C. and 0·5260 H_2O = 11·20 °/₀ H.

(2.) 0·5260 grm. gave 10·75 c.c. gas at 18° C, and 772 m.m. B. KHO column = 173 m.m. = 20 m.m. Hg, equal to 2·30 °/₀ N.

	°/₀ found.	÷ by At. Wgts.	÷ by N = 1.
C	66·60	5·55	33·84
H	11·20	11·20	68·29
N	2·30	·164	1·
O	19·90	1·25	7·62
	100·00		

leading to formula $C_{34} H_{67} N O_8$.

	Theory	
	Of Atoms.	Per Cents.
34 C	408	66·12
67 H	67	10·85
1 N	14	2·26
8 O	128	20·74
	617	

Nitrited Phrenosine.—$C_{34} H_{66} (N O_2) N O_8 + H_2 O$, 10 grms. of the substance just analysed were finely powdered, and added little by little to about 300 c.c. pure $H N O_3$, time being allowed for each portion to assume a gelatinous swelled condition before a new one was added. After all the phrenosine had been added, a thick paste resulted, with some tendency of separation of a solid towards the surface. No red fumes were given off during the operation. The bottle containing the mixture was placed in cold water during 14 hours, at the end of which time the air space in it had become filled with fumes of nitric peroxyde. The bottle was filled up with water, when the oily or gelatinous product immediately assumed a hard waxy consistence; it was filtered, washed with much water, pressed, and dried under air pump. Though kept in the dark, it gradually assumed a strong reddish colour, became hard and brittle, and could easily be powdered. The powder was yellow. It was soluble in hot ether.

Analyses.—Substance dried in air pump, not by heat.

(1.) 0·3977 grm. gave 16·5 c.c. gas at 18° C. and 764 m.m. B. K H O = 21 m.m. Hg, equal to 14·8 c.c. normal = 4·65% N.

(2.) 0·4394 grm. gave 0·960 grm. $C O_2$ = 59·58 % C and 0·3927 grm. $H_2 O$ = 9·93 % H.

	Found	Calculated for $C_{34} H_{66} N_2 O_{11}$
C	59·58	60·17
H	9·93	9·58
N	4·65	4·12
O	25·84	25·49
	100·00	99·38

By the process of nitration the nitrogen has at least doubled, and the oxygen has greatly increased. The nearest hypothesis compatible with the analyses leads us to the conclusion that an atom of H has been substituted by $N O_2$, and an atom of oxygen been added besides, or an atom of water, so that we obtain either $C_{34} H_{66} (N O_2) N O_8 + O$, or $C_{34} H_{66} (N O_2) N O_8 + H_2 O$. The unquestionable fact of the entrance into the molecule of phrenosine of a molecule of $N O_2$ enables us approximately to fix the molecular weight as that given; in any case it could only have to be doubled or trebled, but not otherwise much changed in its proportions.

Phrenosine and Bromine. Mono and Dibrominated Phrenosine.

Phrenosine of the formula $C_{34} H_{67} N O_8$, same preparation as in the previous experiment, when exposed to bromine vapours becomes yellow and combines. The product is soluble in ether and in hot alcohol, and more soluble in cold absolute alcohol than the pure phrenosine.

When phrenosine is thrown into ether containing some Br there is a slight evolution of heat, and the phrenosine is immediately and entirely dissolved. A red solution remains which is not made colourless by powdery phrenosine in excess. The ether solution evaporated leaves a yellow thick residue, which is entirely soluble in warm absolute alcohol, and from this deposited on spontaneous evaporation. The ether solution when boiled with water seems decomposed; the body becomes white and flaky, and H Br is evolved. The products of the brominating process, after purification, when deflagrated with nitre and caustic soda, yield much Br to reagents.

10 grm. of the above purest preparation (ox) were finely powdered and added little by little to about half a pint of ether, coloured with Br; a little Br was added as often as the solvent action upon the phrenosine became slower. Finally a brilliant solution was obtained. To this about an equal volume of water was added, whereby a gelatinous precipitate was produced. Caustic potash was next added and the mixture shaken till the colour of Br was destroyed and the solution was alkaline. After some repose the mixture consisted of three layers, the one uppermost being ethereal and clear, the second one containing the gelatinous body in suspension, and the third being a watery solution of K Br and excess of alkali. The ethereal layer was syphoned off, washed with water, and then allowed to evaporate spontaneously. A homogeneous white mass was left, which was dried under the air pump over $H_2 S O_4$, light being excluded.

Analysis.—0·7205 grm. were fused with caustic soda and nitre, and dissolved in water; silver nitrate in excess was now added and then nitric acid in excess. The mixture was heated to boiling, and the Ag Br filtered and washed, and weighed 0·2400 grm. equal to 14·17 °/₀ Br.

A monobrominated phrenosine requires above 11 °/₀ of Br; a mixture of one molecule of monobrominated with one molecule of dibrominated phrenosine

$$\left.\begin{matrix} C_{34} H_{65} Br_2 N O_8 \\ C_{34} H_{66} Br\ N O_8 \end{matrix}\right\}$$

requires 16·3 °/₀ Br. The above product is therefore such a mixture, in which the monobrominated body prevails. The reaction with Br is thus shown not to be simple, but to yield at least three bodies; of these the gelatinous body has not yet been any further analysed, as its isolation and purification is a matter of extreme difficulty. Nevertheless, experiments with large quantities of material promise interesting results.

Phrenosine and Iodine.—An ethereal solution of iodine was not discoloured by powdery phrenosine (same preparation as used with Br), and the phrenosine did not dissolve. After six weeks' contact the ethereal solution was allowed to evaporate spontaneously and left no residue. Therefore, under these circumstances phrenosine and iodine do not react upon each other.

Phrenosine and Sulphuric Acid in Alcohol.—10 grms. of the preparation $C_{34} H_{67} N O_8$ were dissolved in $\frac{2}{3}$ litre of hot spirit of 85 °/₀

and 8 c.c. of oil of vitriol added. The mixture was gently boiled for one hour and became coloured red. When allowed to cool it curdled immediately to a mass of fine microscopic crystals. These were removed, pressed, and dried, and are named hereafter: *first deposit.* The filtrate from this was evaporated and furnished *the second deposit*; and the filtrate from this, on much concentration, yielded *the third deposit.* The mother liquor was very acid, and contained much sulpho-vinic acid, as was proved by treatment with baryta, crystallisation, &c. It contained no body in solution which reduced alkaline copper-solution.

Analysis of first deposit:—(1.) 0·4690 grm. dried at 80° C gave 1·1142 grm. $C O_2 = 64·791$ % C, and 0·4676 grm. $H_2 O = 11·077$ grm. H.

(2.) 0·5466 grm. gave 8·6 c.c. gas at 0°C and 760 m.m. = 1·96 % N.

	In 100·.	÷ by At. Wts.	÷ by N = 1.
C	64·791	5·399	38·55
H	11·077	11·077	79·12
N	1·960	·140	1·00
O	22·172	1·385	9·89

leading to formula $C_{39} H_{79} N O_{10}$, a formula which approaches remarkably to some of the myelines, phosphorus deducted. Under the influence of heat the body behaved like the original phrenosine. It seemed to be more soluble in ether than the original phrenosine, and was better crystallised. The action of sulphuric acid therefore seems to be in the direction of the removal of the nitrogenous nucleus. But it is evidently very complicated, for while the first deposit was soluble only in hot alcohol, the third one was entirely soluble in cold absolute alcohol, while the second deposit observed an intermediate solubility. Several bodies are therefore produced, which, though they crystallise, can be separated only by operations on much larger quantities than were at my disposal.

Phrenosine and Sulphuric Acid in Water.—About 1 grm. of phrenosine was placed in cold water and boiled until diffused; 5 grm. of $H_2 S O_4$ were now added, so that the whole solution was about 50 grm. The mixture was boiled. The phrenosine coagulated at first, and on continued boiling changed in appearance, contracted, and floated on the surface. The insoluble matter was collected on a filter and washed. (White product; it gave no reduction with cupric solution.) The solution was boiled with $Ba C O_3$ until neutral, filtered and evaporated to near dryness, and deposited a white scaly matter. It had no taste, and particularly no sweet taste, and was not syrupy. It reduced cupric oxyde, the suboxyde falling in yellow flakes, different from that produced by sugar; a secondary deposit settled on yellow suboxyde. It gave no reaction with iron chloride or gold chloride. Caustic potash and heat made the solution slightly coloured yellow, but not brown, and there was no smell of caramel, but on cooling a darkish precipitate occurred. This reaction also is evidently too complicated to be satisfactorily studied on small quantities.

Reducing body.

Phrenosine and Hydrochloric Acid.—About 1 grm. was placed in a flask with H Cl and heated; it frothed a good deal and formed a red solution, the phrenosine swelling greatly. The swelled mass isolated and washed with water was found for the most part soluble in cold absolute alcohol. This solution evaporated to a low bulk deposited in

the form of microscopic needles, which when dry fused about 100° C, and became brownish at 125°; on cooling they were like wax, evidently a body differing greatly from the original phrenosine. The hydrochloric acid solution, after neutralisation tested with Fehling's test, reduced the copper, but unlike sugar, the cuprous oxyde falling in flakes, as in the reaction with H_2SO_4. Possibly there are here two bodies of which one flaky causes the Cu_2O to assume the flaky shape.

Product reduces copper test.

Action of Heat upon this Phrenosine.—0·5290 grm. lost on drying between 18° and 70° C. 0·0084 hygroscopic water, and the remaining 0·5206 grm. were considered as dry substance. After two hours' exposure to a heat of 97° C. in a large air-bath it became slightly yellow, and had lost 0·0016 grm. After three hours' exposure to 101° it had remained of the same colour and weight. After two hours' exposure to 145° it had become very dark, almost black in colour, and superficially fused, and had lost 0·0080 grm. After heating to 158° during three hours it had become thoroughly fused, and of a reddish almost transparent aspect; it had lost 0·0120 grm. in weight. After four hours at 177° C. it had become blacker and less transparent, and gave out a faint odour of burnt meat; it had lost at this stage 0·0317 grm. Thus the phrenosine had lost in four stages 0·0533 grm., or 10·2 % in weight. Taking atomic weight of $C_{34}H_{67}NO_8$ as 617, this loss, considered as a loss of water only, amounts to 3·5 atoms H_2O very nearly. After cooling it was hard and brittle. On boiling a piece in absolute alcohol only a trifling amount of matter dissolved, colouring the solution slightly yellow. On the other hand, it readily dissolved in ether; the solution had a dark reddish brown colour.

Fusion over ga flame.

A little of the same phrenosine was heated in a test tube over the naked flame. It fused, turned dark, and evolved water with ebullition, similar to sugar passing into caramel. The water which condensed in the upper part of the tube had an acid reaction and reduced copper solution. The fused matter became hard on cooling, was but slightly soluble in boiling alcohol, but readily soluble in ether; from the latter solution it was reprecipitated by alcohol.

Phrenosine and Chromic Acid.—Trituration with an equal bulk of crystallised chromic acid produces a mass which, soft at first, and smelling of butyric acid, becomes soon solid, and afterwards can be powdered. Is easily soluble in cold absolute alcohol, and after standing in a beaker for a few days a gelatinous cake is formed. (Chromate of phrenosine?) This when heated gives out fumes, and then suddenly deflagrates, sometimes throwing particles about, leaving chromic oxyde. Boiled with water it forms a plastic mass on the water, which after washing is partially soluble in alcohol, partially in ether; another part is insoluble in both solvents, and consists of a compound of chromic oxyde with some organic matter.

Phrenosine dissolved in glacial acetic acid with the aid of a little heat, is easily attacked by crystallised chromic acid added to the solution. Much gas is evolved, the solution becomes green, and on cooling deposits some product in flakes. Water added to this solution produces a copious precipitate of dark green flakes.

Phrenosine and Alkaline Permanganate.—Phrenosine, boiled with alkaline permanganate, decolourises the latter; green at first, the liquid becomes brown with manganic oxyde. Flakes of decomposition products are formed, and the liquid remains turbid.

H_2O.

Phrenosine and Lead Peroxyde.—Phrenosine triturated with lead peroxyde produces no reaction; none on boiling with water or dilute H_2SO_4.

Ba $H_2 O_2$.
Pb acetate.

Reactions with Water, Alcohol, Caustic Baryta, Lead and Copper Acetate.—Boiled in *water* it swells, and forms, with the aid of agitation, a thick white paste. Baryta water added to this, while hot, forms an immediate curd, which can be filtered, but not washed, as water causes it to swell and filtration ceases, and the rest of the fluid becomes clear. Later, the white flocculent precipitate settles. In the watery solution lead acetate forms a thick curd, which contracts when the mixture is boiled, and is suspended on the top of the fluid or settles. This has smaller bulk than the baryta precipitate; it can be washed with cold or hot water for some time, during which it loses lead. In alcohol of 85 °/₀ phrenosine dissolves completely on heating. In this solution a few drops of baryta water cause an immediate white flaky precipitate. By a sufficient amount of baryta water, added if need be after filtration, a point is reached where baryta water ceases to produce a precipitate. The liquid on cooling sheds a precipitate, soluble on boiling, being a small portion only of the substance originally employed. Alcoholic lead acetate also causes an immediate copious white precipitate in the hot alcoholic solution, insoluble on boiling; another precipitate after filtration, until the acetate leaves the liquid clear. The filtrate deposits some white matter on cooling. [This reaction is very curious, considering the mode of preparation.] Boiled in water, and treated with cupric acetate, it yields a copious light blue coagulum. This remains bluish green during washing, and does not swell.

Alcohol Ba $H_2 O_2$.

Pb acetate.

Cu acetate.

Bearing of the Lead and Copper Compounds during Washing with Water.—By prolonged washing with cold water they always yielded clear solutions, which, with ammonium sulphide, gave a brownish colour, but no precipitate on standing. This showed that the compounds were a little soluble in water, and like the kephaline compounds coloured, but not decomposed by $N H_4 H_2 S$.

Pb salt.

A quantity of lead salt, prepared by acetate from phrenosine swelled in water, was washed for two days, the filtrates containing metal to the last.

0·5160 grm. dried at 100° (at which T. the salt was pasty) gave 0·0396 grm. $Pb S O_4 = 5·24$ °/₀ Pb.

Cu salt.

A quantity of copper salt, prepared exactly like the lead salt, from 1 grm. of dry phrenosine, after washing during two days, had lost so much substance that only 0·859 grm. of dry compound were obtained.

0·4592 grm. gave 0·0364 grm. Cu O, equal to 6·32 °/₀ Cu.

These percentages of Cu and Pb do not admit of any theoretical explanation at present.

Indefinite Cerebrine Preparations, showing Peculiar Bearings of this Substance by itself, in Mixtures, or with Reagents.

Solubility in cold ether.

(1.) *Cerebrine (Ox; third portion of No.* 12, *before Stearoconote).*—This was dissolved in hot benzole, and precipitated by alcohol of 85 °/₀, washed therewith and dried over $H_2 S O_4$. It was placed in ether, and shaken therewith repeatedly at the ordinary temperature; it became somewhat flaky in the ether. The ether extract, on distillation, left a few milligrammes of matter, which was soluble in hot alcohol, but deposited from it, on cooling, (cerebrine, myeline, or kerasine?).

Solubility in boiling ether.

The cerebrine thus extracted with cold ether was now boiled in a great excess of ether, and filtered hot. From the filtrate a few unweighable particles separated on cooling. The filtrate was now distilled until it left a very small residue; this deposited on cooling a minute quantity of white

particles, soluble in hot alcohol, apparently cerebrine. Indeed, the extracted matter was so small in quantity that the extraction with ether was not continued. The cerebrine was analysed.

Analyses :—

Analyses.

Dried at 60° to 70°.

(1.) 0·4423 grm. gave 1·0710 grm. CO_2 = 66·04 °/₀ C, and 0·4349 grm. H_2O equal to 10·92 °/₀ H. C. H.

(2.) 0·3736 grm. gave 5·6 c.c. N normal, equal to (after correction for a trace of nitric oxyde) 1·84 °/₀ N. N

(3.) 1·8706 grm. fused with nitre, &c., gave 0·0184 grm. $Mg_2P_2O_7$, equal to 0·27 °/₀ P. P.

The presence of P having been previously ascertained by qualitative testing only, its amount was expressly estimated on a large quantity of material.

Summary :—

	°/₀ found.
C	66·04
H	10·92
N	1·84
P	0·27
O	20·93
	100·00

If we neglect the P, the other figures lead to an empirical formula $C_{42}H_{83}NO_{10}$, which is not greatly altered if we deduct with the phosphorus a quantity of the other elements corresponding to myeline.

This experiment is stated only because its results are so consonant to the experience of former observers; the substance repeats the peculiarities of Couerbe's cerebrote, Frémy's cerebric acid, Gobley's and Thompson's cerebrine, Bibra's cerebric acid, and yet is unquestionably impure cerebrine, that is to say, a peculiar cerebrine with a large number of carbon atoms, to which a small quantity of myeline obstinately adheres, so that it cannot be removed by cold or boiling ether applied in any quantity. *By further experiment it was found that the myeline can be removed from the cerebrines only by dissolving them in hot absolute alcohol, allowing the cerebrine to deposit on cooling, and thereby keeping the myeline in solution. By repeating this process sufficiently often, cerebrines free from phosphorus are ultimately obtained.* Mere washing or boiling with ether does not remove the whole of the myeline, because this body is very little soluble in ether, and because it is probably mechanically enclosed by the cerebrine.

The bulk of the foregoing preparation was unhappily lost by an explosion and fire which occurred during the progress of its purification.

c. Sub-Group of Kerasines.

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Kerasine.—$C_{46} H_{91} N O_9$.

This body belongs to the group of substances resembling cerebrine and phrenosine in general characters, but differing from it by well defined peculiarities, particularly by its late deposition from alcoholic solution, the form of its crystallisation, and its ultimate composition.

Mode of Isolation.—The ox white matter, which had been extracted with ether, and dried, was pulverised and dissolved in hot absolute alcohol, the solution was decanted from the fused mass of stearoconote and kephaline which formed, and allowed to deposit the dissolved matter by cooling. The deposit formed after the first hour was isolated, as was also another which formed after the second hour, a third gelatinous looking precipitate formed over night, and from this the absolute alcohol solution was filtered. On standing for a few days in stoppered bottles this solution deposited a gelatinous membranous mass, mainly consisting of *kerasine*. This was removed by the filter, and the filtrate which contained much *myeline* in solution was treated with $Cd Cl_2$. The white bulky precipitate of myeline $Cd Cl_2$ was filtered off, washed, exhausted with ether, and further treated as has been described elsewhere. It may be mentioned here that the principal bulk of myeline is obtained in this manner and at this stage. The alcohol filtered from the myeline $Cd Cl_2$ precipitate after concentration deposited a mixture of $Cd Cl_2$ salt, cholesterine, cerebrine, and kerasine, the latter two being present in very small proportions.

Mode of Purification.—Kerasine obtained as above is a soft white gelatinous mass, consisting of larger and smaller balls, which under the microscope are seen uniformly to consist of wavy masses of needles so thin that it may be said they possess only one diameter, namely, length. The gelatinous state is apparently entirely due to this peculiarity of the fine needles enclosing a large amount of alcohol. No amorphous matter whatever is seen mixed with it, but here and there a few rosettes of cerebrine, strikingly differentiated from the kerasine.

Purification by recrystallisation.

The whole of the kerasine was dissolved three times in boiling absolute alcohol, and after cooling to crystallisation washed and pressed free from mother liquor. This after the third operation was free from myeline, as shown by the absence of reaction with $Pt Cl_4$, $Cd Cl_2$, and Pb acetate, which give precipitates in even dilute solutions of myeline. The solution of kerasine was also free from myeline, $Cd Cl_2$ giving no precipitate with it.

Fractional crystallisation.

The kerasine was now dissolved in a fourth quantity of pure absolute alcohol, and allowed to crystallise. It was found by microscopic examination that after three hours much kerasine in wavy crystallised masses, but no cerebrine in rosettes, had been deposited. The crystals were consequently isolated, pressed, again recrystallised, collected on a filter, and dried in vacuo. This preparation only (1267) was analysed with the result stated below.

After this preparation had been removed, at the end of the third hour, from the mother liquor, the latter, on standing, deposited a mixture of much kerasine in wavy needles, with some rosettes of cerebrine. This mixture could by recrystallisation not be completely separated. A third ultimate precipitate seemed to consist mainly of cerebrine, with much kerasine, which could also not be purified by mere recrystallisation. These preparations were therefore put aside for future treatment (1267 ; 1268 ; 1269).

Properties of Kerasine.—Easily soluble in hot absolute alcohol, without leaving a trace of stearoconote. With $H_2 S O_4$ and sugar gives a purple test, which is mainly due to the production of purple particles, and not or only little to the production of a purple solution. Shrinks on drying by loss of enclosed alcohol to a very small bulk, and then becomes waxy, semitransparent, hard and horny. When quite dry is easily powdered, yielding a perfectly white powder. Easily dried at 100° C without any change.

Analyses.—(1.) 0·3670 grm. gave 0·9210 grm. $C O_2 = 68·446$ °/₀ C and 0·3764 $H_2 O = 11·395$ °/₀ H.

(2.) 0·5470 grm. gave 8·5 cc. gas at 18° C and 770 mm. B. K H O column = 16 m.m. Hg equal to 7·6 cc. N normal = 1·738 °/₀ N.

Summary :—

	°/₀ found.	÷ by At. Wts.	÷ by N = 1.
C	68·446	5·704	46·0
H	11·395	11·395	92·0
N	1·738	0·124	1·0
O	18·421	1·151	9·2
	100·000		

leading to formula $C_{46} H_{91} N O_9$. This formula is given with all reserve. The analyses are not controlled by repetition, the molecule is with difficulty only determined by one atom of nitrogen united with so much carbon as to represent 46 atoms. But the purity and uniformity of the substance must be insisted upon. There is probably a large quantity of it contained in the cerebrine matters, which are soluble in boiling alcohol, and contain no phosphorus. The third deposit indeed is always greatly gelatinous and iridesces blue like kerasine. But to effect a complete separation of these substances is a matter of great difficulty, and we must therefore direct our attention at first upon obtaining pure substance at all, by manipulating large quantities of products, then the study of the pure bodies will undoubtedly lead to effectual methods of complete separation.

Experiment with reference to Otto's Cerebrine.—The question was whether by addition of baryta-water to an alcoholic solution of mixed cerebrine bodies the cerebrine, phrenosine, and kerasine could be precipitated, and the cerebrine free from nitrogen, described by Otto, could be obtained. Some crude cerebrine mixture, fully extracted with ether, was used for the experiment. The mixture was obtained from both (ox) white and buttery matter, by ether, which deposited the cerebrine, &c., on cooling in the shape of a disc. All myeline was now extracted by dissolving the matter thrice in hot absolute alcohol and filtering the deposit. The deposit was again dissolved in hot absolute alcohol and tested as follows.

Experiment showing that Water added to this Solution produces no Precipitate while hot.—A portion of the solution was mixed gradually with hot water until it became permanently turbid, which required more than one-fourth the bulk of the alcohol; no precipitate ensued. On cooling the solution set into a gelatinous mass of hydrated cerebrine, &c. This proved that the water to be used in the baryta water in the following experiment could not be instrumental in producing the precipitate.

Experiment with Baryta-Water.—The bulk of the hot solution was now treated with cold baryta water, added drop by drop as long as a precipitate formed. The precipitate immediately on formation was white and granular, but after subsidence caked together and became a soft viscous flowing mass like stearoconote, which adhered to the glass. The liquid was decanted and filtered hot.

The Precipitate was a plaster-like mass, which set on cooling into a hard solid mass, and after drying was easily powdered. It was a baryta compound, or mixture of baryta compounds, and on analysis was found to contain much Ba.

The Filtrate deposited on cooling a trifling amount of white flaky matter, which was isolated by filtration, washed with alcohol, dried, and analysed for nitrogen by combustion with copper oxyde with all precaution. It contained two per cent. of N. One analysis absorbed all the material, which amounted only to a few per cents. of the matter which had been precipitated by baryta water.

What is proved by this experiment is that after complete precipitatation by baryta-water the solution does not deposit a cerebrine body free from nitrogen. The experiment is not entirely paralled to that of Otto, who used, as he says, excess of acetate of lead with his brain-pulp, which was not used in my case. But it is not stated by Otto how this excess of lead-salt was admeasured or ascertained, and it is not easy to see how, if it had actually been used, any cerebrine whatever could have been obtained by boiling the insoluble product of the lead-treatment in alcohol. Further, Otto does not state the method by which he proved the absence of nitrogen from his product. I am therefore inclined to think that he overlooked the nitrogen, perhaps in testing his substance only qualitatively by the potassium test.

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§ Summary.

The foregoing research is a contribution towards a system of *chemical statics* of the brain, and occupies itself with brain matter as a whole, without separating white from grey matter; it isolates and scrutinises the several chemical constituents of nerve-matter, and endeavours, by the study and consideration of the peculiarities of each, and their combination, to obtain sufficient insight into normal and abnormal chemical functions to enable us in time to guide or to correct them.

It is thus found that this apparently so simple nerve-marrow is a compound and mixture of a large number of heterogeneous principles arranged in such a manner as to vanish completely from appearance as chemical individuals; the compounds so interpenetrate each other that the resulting material is apparently homogeneous, during life completely so, when seen with high powers of the microscope, and although in death the homogeneity partly vanishes, yet even the appearance of the cylinder axis cannot be utilised chemically at present, and the isolation and recognition of any ingredients is entirely dependent upon most circumstantial chemical proceedings.

During these proceedings the first striking fact which meets the inquirer is that nerve-matter contains abundance of water. This, in conjunction with the peculiar manner in which the water is contained, engenders a mobility of ultimate particles within certain limits of movement. It also gives penetrability by liquid diffusion, while excluding porosity and its capillary effects; by which means a ready nutrition by diffusion in one direction, and ready cleansing from the effete crystallisable products of life in another, are ensured. Consequently the brain as a whole is essentially made up of colloid matter, and may be compared to a colloid septum, on the one side of which is arterial blood and cerebrospinal fluid of the ventricles, on the other side however, is cerebrospinal fluid of the arachnoideal space and venous blood. It follows from this that the large amount of water present in the brain, is not there so to say mechanically only, like water in a sponge, and capable of being pressed out mechanically, but is chemically combined as colloid hydration water, or better, *water of colloidation.*

All soft organs of the body contain about three quarters of their weight of colloidation water, fixed within the organised limits of cells and fibres of all kinds. Cells and fibres are thus apparently not very different in respect of their mechanical condition from brain matter. Indeed the difference is less in the manner of the condition than in the agents by means of which the condition is brought about. It is in effect mainly by the number and nature of these agents that brain matter is distinguished from other colloid tissues. It contains a considerable amount of an albuminous base. Whether this is distributed in the form of sheaths to fibres of nerve marrow, or whether it is laid into hollow spaces between the fibres, and acts as a cement, whether it is mixed or combined chemically with the rest of the matters constituting nerve marrow, cannot be here discussed. It may be present in all forms, but does not seem to be present in a liquid unattached form, as in serum. It appears that the bearing of soluble albumen when placed in presence of some of the peculiar brain matters changes from that which it ordinarily observes, no doubt by an influence of these brain matters amounting almost to combination. The bearing of albumen in the brain being thus seen to be governed by the matters peculiar to the brain, the present research has in the first instance not been directed upon the condition and nature of the albumen in the brain, but upon the peculiar matters which seem not only to govern the albumen, but by their manifold chemical affinities assist prominently in producing its singular mechanical arrangements, chemical function, and sensory, and volitional action and reaction.

It is not asserted that these matters, or any of them, occur exclusively in brain matter, but being present in the nerves, they consequently can be extracted from all sensitive or contractile tissues. They, or some of them, also occur in aggregations of loose cells, such as the blood-corpuscles and pus-corpuscles; others are present in serum, and others again in secretions, such as bile; but the quantities in which these bodies are met with in parts and matters other than those of the nervous system is very far less than that in which they occur in the nervous system.

The great quantity of these matters occurring in the brain forms three groups; the members of one contain five elements, amongst which is phosphorus; hence they may be termed PHOSPHORISED BODIES. The members of the second group contain four elements, amongst them nitrogen, but no phosphorus, and therefore are termed NITROGENISED BODIES. The members of the third group contain only three elements, carbon, hydrogen, and oxygen, present also in the other two groups,

but neither phosphorus nor nitrogen, and may be termed OXYGENISED BODIES.

The group of the PHOSPHORISED bodies contains the phosphorus in the form of phosphoric acid, combined proximately with glycerine, so that by chemolysis they all yield glycero-phosphoric acid, but they differ in the manner in which they contain the nitrogen, and the acid radicles which constitute the great bulk of their substance, and according to these differences must be divided into sub-groups. We thus obtain the sub-groups of the *kephalines*, *myelines*, and *lecithines*.

Of these the *kephaline* sub-group, itself hitherto unknown, includes members which contain the nitrogen in either one or two forms, one being either choline or neurine, another hitherto unknown; and they contain the fatty acid radicles also in forms with which chemistry is at present unacquainted, and the members of this sub-group further vary in the amount of oxygen which they contain in a manner so as to be sharply characterised thereby. This variability of the constituent oxygen may be transitional, but must not be confounded with that remarkable reaction of the bodies of this group which I describe as their oxydisability.

The *myeline* sub-group, also new, contains the nitrogen in two forms, of which one is choline, the other amide in a fatty acid radicle. The fatty acid radicles vary, and are mostly new forms, some known forms. The members of this group consequently vary in carbon, hydrogen, and oxygen; little in nitrogen, never in phosphorus. They are not oxydisable after the manner of kephaline, though there is an oxy-myeline after the manner of oxy-kephaline. They are the least soluble of the entire group, the least decomposable, and stand the highest temperatures, being unchanged by fusion at a heat above the boiling point of water.

The *lecithine* sub-group, well known from the chemistry of eggs, is only with difficulty evolved from the brain, on account not only of the many stages of the processes necessary for their isolation, but also on account of a prominent feature of its members, namely, their readiness to decompose when in the anhydrous state. This tendency to apparently spontaneous lysis into proximate nuclei prevents the inquirer fixing properties and varieties with the same precision as in the previous groups; but it furnishes a valuable key to the explanation of many changes in the sick body, which may arise, or have been proved to arise, from their decomposition.

The chemical characteristics of these sub-groups may be summarised thus: the kephalines possess the tendency to be oxydised, oxydisability; the myelines are not easily changed by any agent or influence, and possess therefore stability; the lecithines easily fall to pieces, they are afflicted with lability[1]

In language more technically chemical: the *kephalines* have on the outside of their molecules free affinities for oxygen; this gas they bind in several ways; when the oxygen, combined with a molecule has attained a certain quantity, the avidity (intensity of affinity) of the molecule increases to this extent, that it monopolises all available oxygen to itself until the limits of its oxydisability (at present unknown) are attained; until its free affinities are satisfied. Until then the rest of the molecules, if the supply of oxygen be insufficient to oxydise all to the same point, are not oxydised. The kephalines, however, are not in a state of atomic tension, and therefore do not fall to pieces so easily as the lecithines, but require for lysis the influence continued for some time, of powerful extraneous affinities in the presence of water and heat.

The *myelines* have no apparent free affinities for oxygen; they are not affected by heat to and above boiling water except to the extent of fusion; their atoms are not in a state of chemical tension, but require

for vibration into permanent decomposing distances the influence of strong external affinities, water and heat.

The *lecithines*, however, are in a state of great atomic tension, and therefore slight external affinities or dissociating impulses suffice to effect their decomposition. Such a slight impulse is the attraction of absolute alcohol for their fatty acid radicles in the absence of external water. The water given out by the alcohol in becoming ethylic ether serves to enable the radicle of glycerophosphoryle to become glycerophosphoric acid, and to remain in combination with the choline evolved.

The presence of water diminishes the number or avidity of affinities in in all these sub-groups ; it combines itself with these bodies in a peculiar manner, by which they show their character as colloids, and it afterwards dissolves them in a peculiar and imperfect manner. When this hydric colloidation is at a maximum, the dissociating faculties of the members of these sub-groups seem to be at a minimum. The watery solutions do not decompose in stoppered bottles for many months ; only after six months some specimens so kept showed signs of decomposition or putrescence. It seems therefore that water satisfies some of these affinities, and what is most remarkable, its influence increases and diminishes with the mass which is present and capable of acting, so that it displaces, when in quantity, other combinants, but when these other combinants prevail, water is itself displaced, and the colloid state instantly disappears. In the dilute watery solutions of the phosphorised bodies therefore almost every reagent soluble in water, when added in a certain excess, produces a precipitate, which contains the reagent in combination. But when water, or any watery solvent capable of dissolving the combined reagent, is brought in contact with the compound, the compound immediately dissociates ; the reagent passes into the water, *pari passu* as the phosphorised body passes into the hydrated colloid state, and if the influence of the water is continued by renewal, the process terminates by a complete separation ; the phosphorised body is again free and pure, and swells and dissolves as at first.

The reagents with which the phosphorised bodies are thus able to combine, and from which they are dissociated by water, are acids, alkalies, and salts. The phosphorised bodies therefore possess alkaline affinities (for acids), acid affinities (for alkalies), alkaloid affinities (for salts) ; all these affinities are overcome by water in quantity, but the affinities for water are overcome by some metallic oxydes, such as of lead, copper, manganese, iron, and even to a slight extent by lime and potash ; these latter compounds are dissociated only by strong mineral acids, and the compounds can then be dialysed out. All other combinants separated by water alone can be completely removed from the phosphorised substances by dialysis on a diaphragm of parchment paper.

We have therefore here a diversity of affinities such as is not possessed by any other class of chemical compounds in nature at present known ; and the exercise of these affinities being greatly influenced by the mass of reagent, and the mass of water which may be present, the interchange of affinities may produce a perfectly incalculable number of states of the phosphorised and consequently of brain-matter. This power of answering to any qualitative and quantitative chemical influence by reciprocal quality or quantity we may term the state of *labile equilibrium ;* it foreshadows on the chemical side the remarkable properties which nerve-matter exhibits in regard of its vital functions.

From this it also follows that nerve-matter (if only as characterised by the phosphorised bodies) must yield obedience to every, even the slightest external chemical influence, which may reach it by way of the blood. It must take up metals, acids, salts, alkalies, and alkaloids presented by the blood ; it can retain only oxydes when the serum is again

free from the combinants; a watery serum will wash the brain, a more watery one will make it swell and displace mechanically within physiological limits what it can; a still more watery one will make the brain dropsical and produce all the conditions of mechanical pressure on the brain. All these processes are the necessary consequences of the affinities of the phosphorised substances, and these being known the phenomena could be predicted, if they were not sufficiently known as phenomena, though hitherto destitute of an explanation. Thus the so-called brain-fungus, the continued protrusion of brain-matter through apertures of the skull produced by mechanical injuries, may in certain cases find a physical explanation in simple excessive hydration of the phosphorised (and nitrogenised) principles, producing general intra-cranial pressure.

These few examples show that the acquisition of chemical statics leads almost necessarily and very easily to chemical dynamics of the brain; and these will in their turn furnish data for physiological and pathological conclusions. But these deducive arguments must be most sparingly and cautiously used, until the statics are in a state of perfection and completeness. To argue too far from incomplete data would, seeing the history of biological chemistry during the last thirty years, be a deplorable error.

The NITROGENISED substances of the brain imitate in many respects, but in a lesser degree, the properties of the phosphorised. Thus they have the affinity for water to the extent of swelling to gelatinous masses, but they do not go to the state of apparent solution, and do not pass paper filters under any pressure. They are insoluble in ether, cold benzole, cold alcohol; dissolve in hot benzole or alcohol, and are deposited on cooling almost entirely. Therefore as compared to the phosphorised bodies, their chemical character is slight solubility; from their hydrated colloid state they are also reduced to a more compact and combined one by many acids, alkalies, and salts, and they retain many oxydes in combination. Water has the same dissociating influence upon these as upon the compounds of the phosphorised principles. There are, however, slight differences. The nitrogenised bodies are all firm compounds, and do not easily oxydise or decompose. Their atoms show very little tension; but they possess substitution poles, where hydrogen is replaceable by metalloids, or compound radicals; and these compounds seem more stable, and to admit of isolation to some extent, while the compounds with salts, oxydes, or acids, are so unstable as not to admit at present of quantitative definition.

The members of both the phosphorised and nitrogenised groups are all, as stated, strictly colloid, and do not pass through the septum of a dialyser. In accordance with this they show only little tendency to crystallise under any circumstances; several have not been observed crystallised or crystalline at all, *e.g.*, the kephalines and lecithines; the cerebrines and phrenosines may be just termed crystalline when slowly deposited from alcohol; the myeline and kerasine are undoubtedly crystallised when deposited slowly from alcohol, but the forms are microscopic.

The presence of a trace of water effaces all crystalline forms, and leaves the shapeless colloid. Thus the definition by Graham of colloids, which did not exclude their power to crystallise under limited conditions, is fully borne out by these cerebral principles.

The third group, isolated, but not specially discussed or studied in the research, that of OXYGENISED principles, consists mainly of alcohols with very slight combining power. The most prominent one of these, both as regards quantity and appearance is *cholesterine*. Discovered originally in human gallstones, and erroneously believed to be a fat, this principle received the inappropriate name which hides its signi-

ficance and character. Insoluble by itself in water, it is probably dissolved in brain matter by means of the phosphorised substances. Its bearing is therefore governed and its role determined to a great extent by these matters. But its intrinsic chemical dynamis is probably also independent, to some extent, as its atomic weight is very high. As an alcohol it is monodynamic, and if it did combine with acid radicles naturally would therefore give rise to one class of ethers only. If such compounds exist in the brain, they must be in a state of high atomic tension, and fall to pieces by the mere fact of the application of solvents. These and other questions concerning this class of bodies, belong properly to the chapter of chemical dynamics upon which this research has had no opportunity of entering. The other oxygenised principles have not even been treated statically, and are therefore not disturbed from their historic slumbers.

There is met with at almost all steps of the separation of the ingredients of white matter, a substance distinguished by fusibility and insolubility in boiling alcohol, which by Couerbe was named *stearoconote.* Although not treated of in the present research a few explanatory notes regarding it may properly be given in this place. When white matter is placed into a quantity of hot alcohol, most cholesterine and lecithine and some cerebrine dissolve, but the other phosphorised and nitrogenised matters immediately fuse into a plaster-like mass, which is then practically insoluble in boiling alcohol. There is therefore here an attraction produced by the fused state which removes the solubility of some of the ingredients of the mass in alcohol. When the principal matters soluble in ether, *i.e.*, the kephalines, are now extracted, the fusibility is diminished, and some of the cerebrines dissolve more freely in hot alcohol. Stearoconote, however, still forms, and is now a reaction mainly of myeline and the cerebrines. When all myeline is separated from the cerebrine group of bodies, their power of forming stearoconote is depressed to a minimum, but not entirely removed. Myeline by itself also retains this tendency of forming a fused mass insoluble in boiling alcohol, which in the text has been termed a tendency to stearoconotise. The phenomenon seems to be compound, and not simple, and to consist in essence of a molecular change without attendant change in quantities of atoms of elements. For I have found that some stearoconote, free from phosphorus could by treatment with benzole and little hydrochloric acid be transformed into cerebrine, which on elementary analysis was found to have the same empirical composition as the stearoconote from which it was made. In this case no separation of a base from the cerebrine could be proved to have taken place. In other cases it was found that the stearoconote obtained its solubility in alcohol without the intervention of acids, by standing in benzole, in which it is easily soluble. Again, in other cases it was probable that the stearoconote was really a compound of some nitrogenised body with a mineral base, and bodies resembling the stearoconotes in some respects, but not in all, were produced by adding bases to solutions of the cerebrine series. On the whole, then, "stearoconote" does not seem to be a chemical individual differing from the other bodies described, but a function of several bodies, which pass into that state by a molecular change, which change can be made to retrograde into the originally soluble condition.

The function is a result of chemical and physical influences acting simultaneously (alcohol and heat) and is apparently not obtained by either influence separately. Stearoconote seems, therefore, a product, and not an educt; but it is indirectly important, as showing that there is some peculiar attraction or mutual influence between the bodies of the two great groups. In white matter where those brain-principles are all

mixed together nearly in their original proportions (only lecithine being much diminished in quantity) the above influence has its highest intensity. The function diminishes with increased separation, *i.e.*, increased purity of educts. It is entirely lost in some of the educts, when they are in the pure state; thus pure kerasine could by no means be made to yield any stearoconote. But it remains with others, particularly myeline and cerebrine in the pure state, and is evoked when they are subjected to the influence of heat in the presence of a quantity of alcohol insufficient to dissolve the whole of the material before it has had time to fuse. Once fused, it seems to resist solution, even after repeated powdering to increase the surface for the action of alcohol, to the extent of being rather destroyed than dissolved.

In the phenomenon just described we observe as the main result diminished or suspended solubility, that is to say, suspension of the lowest form of chemical attraction. In a phenomenon now to be pointed out, we perceive however, on the contrary, the production or elicitation of a solvent power of a kind hitherto quite unknown in animal chemistry. The phosphorised bodies, when combined with metals or metallic salts, in the presence of ethylic ether, and whether dissolved in it or not, cannot be separated from these metals by hydrothion. The dissolved compounds assume the colour of the respective sulphide, and remain dissolved; the compounds insoluble in ether immediately pass into solution when the hydrothion gas is passed through the mixture. Thus, cadmic chloride compounds dissolve with a canary yellow colour; platinic chloride compounds with a dark brown colour; lead compounds with a red or blackish-red colour. The compounds can be partially or entirely precipitated by water, or alcohol, or ammonia, &c. They contain, in addition to the phosphorised body, in the cases of chlorides, chlorine and sulphur, besides metallic sulphide; in the case of metals like lead, metallic sulphide and sulphur. Their complication places them at present beyond the reach of stoechiometric treatment.

When once thus combined with sulphur (or hydrothion) and metallic sulphide, or with these and chlorine in addition, and precipitated and isolated, the phosphorised bodies can by no means ordinarily at hand be again obtained in the free state. They then behave, as they do in solution, somewhat like combinations of mercaptans with metallic sulphides. The compounds with metals of the phosphorised bodies can be decomposed by hydrothion only while suspended in water; the product, a mixture of metallic sulphide with the respective body, must then be washed with water until pure, and the respective body extracted by a suitable solvent.

The obstacles which these extraordinary properties of the chemical principles of the brain throw in the way of chemical procedure are perfectly indescribable. To use a simile from military life, the biologist who attacks his problem in front is beaten off at all points; he can only conquer it by flanking on long and circuitous routes, and by the use of instruments of warfare which are either new or superior to those hitherto in use.

These difficulties increase with every step which leads nearer to the consummation of the purpose, and are greatest at the point where the isolated chemical individuals are to be freed from the last traces of admixed impurity. The very peculiarities of the principles described are mostly negations of the properties ordinarily relied upon as criteria of chemical purity. On this matter I have repeatedly dilated in the text, and given expression to apprehensions as well as to considerations calculated to remove them. As the difficulties arose they were followed out experimentally, one by one, step by step, to the exhaustion of the known means at hand, or of the new means that could be devised in the

time. But it must be left to the future to increase, if possible, both the means of producing, and also the criteria for insuring, that absolute purity of ultimate educts which is the condition of certainty in chemical science.

It is therefore not asserted that in the foregoing research the absolute limits of the subject have anywhere been reached. But it is confidently believed that its entirety has been explored in such a manner that fundamental truths cannot have escaped observation, and that what remains to be done is essentially of the character of detail, which, however vast by multiplicity it may become, will not alter the broad outlines which this investigation has led me to state.

While then there may be some degree of uncertainty as to the absolute purity of some of the principles evolved, there can be none as to their striking individuality ; and, as regards this, the positive evidence of their peculiar and distinctive qualities is so strong that the fact of their not uniformly answering to certain other criteria is, in my opinion, quite insignificant.

Thus the solubilities of kephaline separate it widely from all other brain matters ; its reddish colour and green fluorescence when manipulated in air cause it to be recognised in very dilute solutions. By analysis it exhibits an amount of oxygen exceeding from one third to one half that of other phosphorised matters. By chemolysis it yields not any known fatty acids, but fatty acids hitherto unknown, and a portion of its nitrogen also in forms hitherto unknown.

Again, the solubilities of myeline and of its compounds admit of being used for its easy and complete separation from kephaline on the one, and lecithine on the other, hand. Its positive properties all exclude kephaline and lecithine easily, less easily the bodies of the cerebrine group ; but these are entirely differentiated by benzole, to which they oppose a perfect insolubility.

The separation of lecithine from kephaline must on the face of the processes be complete ; the separation of all kephaline from lecithine is, however, at present not to be absolutely ensured, and the circuitous proceedings necessary to approach the purpose seem to be fatal to the stability of the principle to be purified.

The distinction of individuals is more difficult in the cerebrine group, the members of which are more similar to each other in the properties as yet known. Of the individuality of kerasine there can, I think, be no doubt ; at least if strictly uniform crystallisability be of any value as a criterion. More doubt may be allowed in the matter of phrenosine, where these doubts have led me to a great number of experiments to establish the purity and fix the size of the molecule. As regards the rest of the group the information given is little more than a preliminary pioneering account, valid as far as it goes, but not in any particular of a final nature.

Such information can probably only be secured hereafter by the manipulation of quantities of material on a scale such as no enterprise in biological chemistry has hitherto had the opportunity of adopting. In particular it is just the necessity for the ultimate perfect purity of the principles to be established which creates the necessity for the manipulation of large quantities.

It gives me pleasure to have this opportunity of acknowledging and recording the uniformly assiduous, able, and skilful help which I have received during almost the whole of this research from my assistant Mr. C. T. Kingzett.

App. No. 5.

On the Chemical Constitution of the Brain, by Dr. Thudichum.

III.—ALPHABETICAL LIST OF CHEMICAL EDUCTS (IMMEDIATE PRINCIPLES) AND PRODUCTS (BY CLEAVAGE OR SYNTHESIS), STATED TO HAVE BEEN FOUND IN OR PRODUCED FROM THE BRAIN OF MAN AND ANIMALS; WITH NOTES CONCERNING PROBABLE IDENTITIES AND SYNONYMS.

Note.—The substances marked with an asterisk thus* are believed to be now described for the first time as ingredients in brain matter. The substances marked with dagger † are recognised as mixtures, or lack the characteristics of chemically defined simple bodies, and it is therefore proposed to discontinue the names. The educts include not only normal constituents, but also accidental ingredients.

SYNOPSIS OF NAMES.

1. Acetic acid.
2. Acid, new, containing P and S.
3. Acids, Liebig's, two new.
4. Albumen.
5. Albuminous matters.
6. Alcohol.
7. Ammonia.
8. Amyloidine.
9. *Apomyeline.
10. Arsenic.
11. *Base, new, from kephaline.
12. *Buttery matter.
13. Caseine.
14. †Céphalote.
15. †Cerebric acid.
16. Cerebrine.
17. *Cerebrinic acid.
18. †Cérébrote.
19. Chlorides.
20. Cholesterine.
21. Choline.
22. Copper.
23. Elaine.
24. Elastine.
25. †Eléencéphole.
26. Extractives.
27. Formic acid.
28. *Glyceramine.
29. Glycerophosphoric acid.
30. *Hydrothion.
31. Hypoxanthine.
32. Inosite.
33. Iron.
34. *Kephaline.
35. *Kephaloidine.
36. *Kerasine.
37. *Kephalic acid.
38. Kreatine.
39. Lactic Acid.
40. Lead.
41. Lecithine.
42. Leucine.
43. Leucine homologue.
44. Manganese.
45. Margaric acid.
46. Margarine.
47. Mercury.
48. *Myeline.
49. †Myeloidine.
50. †Myeloidinic acid.
51. †Myelomargarine.
52. Neurine.
53. †Neurolic acid.
54. *Oily, last, matter.
55. *Oleate of ethyle.
56. Oleic Acid.
57. Oleine.
58. †Oleophosphoric acid.
59. Osmazome.
60. *Oxykephaline.
61. Palmitic acid.
62. Palmitine.
63. *Paramyeline.
64. *Peroxykephaline.
65. Phosphates.
66. Phosphorus.
67. *Phrenosine.
68. †Protagon.
69. *Pyrokephole.
70. Silica.
71. Stearic acid.
72. Stearine.
73. Stearoconote.
74. Sugar.
75. Sulphates and sulphur.
76. Syntonine.
77. Trimethylamine.
78. Urea.
79. Uric acid.
80. Water.
81. White matter.
82. Xanthine.

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1. *Acetic Acid* (Müller) obtained by distilling extracts to which lead acetate had previously been added. Therefore probably introduced by reagents, and not proved to be naturally present in brain.

2. *Acid, new, containing P and S*, found by me in human brains, which had been preserved in spirit. The same brains also yielded hydrothion and glycerophosphoric acid, from which it is probable that the sulphur became attached to the phosphorised body as a result of the decomposition of albumen. The observation is important as bearing upon the analytical results of Couerbe, who found sulphur in all his phosphorised brain extracts.

3. *Acids, Liebig's two new*, obtained as baryta-salts, of which one was soluble in alcohol. Not yet identified.

4. *Albumen.*—It is probable that the brain contains soluble albumen, but this has not yet been proved.

5. *Albuminous Matters.*—These remain in the insoluble state after all other matters are extracted from the brain by alcohol. They have been variously sought to be identified with caseine, elastine, syntonine, and others, but proofs are wanting. In particular Lehmann's surmise of the presence of caseine in the soluble form, based upon the observation that watery brain-extracts on heating or evaporation form skins on their surface similar to milk, is now of no value, since I have shown that most phosphorised bodies in the pure state, when heated in watery solution, form these membranes.

6. *Alcohol.*—Obtained from the brain by distillation after death from poisoning with spirit, or from chronic alcoholism, commonly termed delirium tremens.

7. *Ammonia.*—Present in the brain of man and animals ; obtained from ultimate watery extracts, and found in combination with phosphorised bodies in very small quantities only.

8. *Amyloidine* (Virchow).—A matter found microscopically only, and not yet chemically isolated ; a product of certain diseases, particularly locomotor ataxy, in which it occurs in quantity in the spinal marrow. When coloured blue by iodine in the presence and under the influence of sulphuric acid it closely resembles wheat starch in appearance.

9. **Apomyeline.*—Probably a product and not an educt, obtained by boiling the myeline hydrochloride platinic chloride in absolute alcohol. Possibly a dissociation ensues in the boiling solution, in consequence of which a new platinum compound is formed, which contains and yields this body.

10. *Arsenic.*—Found after acute and chronic poisoning.

11. **Base, a green-coloured.*—Obtained by chemolysis of some of the phosphorised compounds ; its platinic chloride salt is soluble in absolute alcohol, and precipitated from this by ether.

12. **Buttery Matter.*—The mixture of kephaloidine, paramyeline, cholesterine, &c. obtained after the first concentration of the alcohol extracts of brains which have deposited white matter. A convenient term for a first crude product.

13. *Caseine* (Lehmann).—Inferred from the formation of membranes on evaporating watery brain extracts. As most of the phosphorised bodies show the same peculiarity, there remains no ground for the surmise, except the finding of potassium albumen in the NaCl solution with which brain has been treated. (See my Manual, p. 86, § 6.)

14. †*Céphalote* (Couerbe).—Mainly kephaline and kephaloidine, mixed with oxy- and peroxy-kephaline, their earthy, alkaline, and metallic salts, and much colouring matter. Stated to contain sulphur, probably erroneously, or if it did contain sulphur the latter was attached only, and not constituent.

15. †*Cerebric Acid* (Frémy). — A mixture of the cerebrine, phrenosine, kerasine class of bodies, with a certain small proportion of

myeline. Nearly the same as Couerbe's cérébrote and Gobley's cérébrine, Liebreich's protagon, and Bibra's cerebric acid. The phosphorised bodies met with in the brain contain from 5 to 20 times the amount of phosphorus which these authors found in their products. Moreover, none of these products could be free from cholesterine and bases.

16. *Cerebrine.*—Term first used by Kühn for a mixture of phosphorised matters and cholesterine, afterwards by Lassaigne for an alcohol extract of nerve substance, not any better defined. The same term afterwards used by Gobley for the mixture described in the previous § 15, containing P. Again applied by Müller to the substance free from P, $C_{17} H_{33} N O_3$. This latter terminology is applied exclusively in the present research.

17. **Cerebrinic Acid.*—Term applied in this research to some nitrogenous compounds of the cerebrine type, containing above 34 atoms of C, and evincing a greater combining power with lead, by means of which they are isolated.

18. †*Cérébrote* (Couerbe).—Mixture of bodies of the cerebrine type with some myeline, from which latter the mixture derives the small amount of phosphorus which it contains. See § 15 above, Cerebric acid.

19. *Clorides of Potassium and Sodium.*

20. *Cholesterine.*—The same as found in gallstones. This identity first announced by Gmelin, first proved by chemical analysis by Couerbe.

21. *Choline.*—Ammonium base, discovered by Strecker as a product of the chemolysis of lecithine in bile. Identical, or nearly so, with neurine.

22. *Copper.*—Normally present, according to my researches, as a base in small quantities in combination with the phosphorised bodies, and also in somewhat larger quantities in the albuminous part of the brain of both man and the ox.

23. *Elaine.*—The solidified form of oleine. Doubtful.

24. *Elastine,* the fibrous tissue which forms the ligamentum nuchæ and the elastic coats of arteries, is supposed by some to be a constituent of the structural connective base of the brain. Lacks definite proof.

25. †*Eléencéphole* (Couerbe). — Berzelius proposed the term *cerebrole,* which was never adopted. Mainly the "last oily matter" of the present research, but believed by Couerbe to be a simple substance. It contains no doubt portions of the greater number of phosphorised bodies, which can be extracted from it. Cholesterine cannot be entirely removed from it. Its oily character is probably derived from the presence of a considerable amount of oleate (and margarate) of ethyle, formed during the processes from decomposing lecithine and perhaps other matters. Stated by Frémy to be oleine, but this is improbable, on account of data to be stated under oleate of ethyle. Gobley and myself never found any oleine.

26. *Extractives.*—The water extract has an agreeable odour of broth, *osmazome* of the older authors. It contains *acids* resembling in bearing those which I have described elsewhere under the name of kreatylic acid (from flesh), and kryptophanic and paraphanic acid (from urine).

27. *Formic Acid* (von Bibra).—Found (in human brains only, Müller) amongst the products of distillation of brain extracts with acid.

28. **Glyceramine.*—Probable product of the chemolysis of kephaline. Possibly only isomeric with Berthelot's product of that name. To be considered as hypothetical.

29. *Glycerophosphoric Acid.*—Product of chemolysis of the phosphorised substances, and therefore a proximate constituent of all of them. First observed by Gobley in Pelouze's laboratory, where the acid had

shortly before been synthetically prepared by Pelouze. Found in diseased brain by Lehmann.

30. **Hydrothion.*—Probably only as a product of physiolysis of the albuminous part of brains kept in spirit ; observed by myself.

31. *Hypoxanthine* (Müller).—Not definitely proved.

32. *Inosite* (Müller) in ox brain, not in the brain of man. Definitely proved and isolated in relatively large quantity.

33. *Iron.*—Always present in combination with some phosphorised bodies, and in the albuminous part.

34. **Kephaline.*—The principal and most remarkable phosphorised ingredient of the brain of man and the ox. Was contained in the céphalote of Couerbe, but missed or neglected by all subsequent inquirers. Occurs in part regularly combined with K, Na, Ca, Mg, Fe, Cu.

35. **Kephaloidine.*—A phosphorised body, very similar in constitution and properties to the former, but differing slightly by being less solid and cohesive.

36. **Kerasine.*—A nitrogenised body of the cerebrine type, free from phosphorus ; crystallises in globules of microscopic needles.

37. **Kephalic Acid.*—Product of the chemolysis of kephaline.

38. *Kreatine* (Müller).—In human brains only.

39. *Lactic Acid.*—Von Bibra.

40. *Lead.*—In fatal cases of chronic lead-poisoning.

41. *Lecithine.*—Discovered in eggs by Gobley, who believed the phosphorised matters of brain substance to consist of lecithine only, although he could not isolate it, and then assumed cerebrine (supposed to be phosphorised) to be present besides lecithine. This erroneous conception has been maintained down to the present time by many authors, who admitted only lecithine, sometimes of two kinds—di-oleyl and di-stearyl lecithine, and entirely neglected the totally different bodies of the kephaline group. According to my research, lecithine is probably present in the brain, but is most difficult to isolate in a pure state ; it decomposes almost entirely during the process of purification from kephaline, with which it is necessarily largely mixed. Occurs combined with K, Na, Ca, Mg, Fe, Cu.

42. *Leucine.*—Not definitely proved.

43. *Leucine-homologue, containing* C_5 (Müller).—Hypothetical ; not definitely proved.

44. *Manganese.*—Accompanies iron in the phosphorised and albuminous ingredients, and is always present in notable quantities.

45. *Margaric Acid* (Gobley).—According to this author, a constant product of the chemolysis of the viscous matter, containing lecithine, from brain ; obtained by Strecker from egg-lecithine. Formulæ and fusing point of acid uncertain, which leaves the question open whether different brain matters do not yield different margaric acids. This acid should therefore not be rashly confounded with a mixture of stearic and palmitic acid. I have not as yet obtained any solid fatty acid from brain-matter which had a fusing point above 52° C.

46. *Margarine.*—Not proved. *See* stearine and palmitine.

47. *Mercury.*—In cases of death from chronic poisoning by mercury.

48. **Myeline.*—This name was applied by microscopists to various extracts of animal tissues and fluids which they examined microscopically, and which showed a peculiar bearing with water (swelling). These extracts no doubt contained mixtures of all the phosphorised bodies, but no single substance was chemically identified. I therefore adopt the

name only for my definite chemical educt, and attribute little diagnostic value to the microscopic myeline forms, so called, which are produced by most or all bodies of the phosphorised groups, and in a certain degree also by the bodies of the cerebrine groups. Occurs combined with K, Na, Ca, Mg, Fe, and Cu.

49. †*Myeloidine* (Koehler). — A phosphorised body, found and analysed by this author as lead-salt; corresponds to one of the myelines of my research in which N : P = 1 : 1.

50. †*Myeloidinic Acid* (Koehler).—Mainly bodies of the kephaline group, with considerable impurities.

51. †*Myelomargarine* (Koehler). — Mixture of products obtained by the influence of sulphuric acid and alcohol and two hours' boiling upon the mixture of the cerebrine group of bodies. Said to be free from nitrogen, but this is not proved.

52. *Neurine* (O. Liebreich).—Product of the chemolysis of the supposed protagon, being actually the ammonium base yielded by the myeline and lecithine groups of bodies, and identical with, or nearly identical with, choline, obtained by the same processes from the lecithine of bile (Strecker).

53. †*Neurolic Acid* (Koehler).—A product of decomposition of phosphorised matters, dissolved probably in oleate of ethyle.

54. *Oily, Last, Matter.*—Convenient term for the semifluid mixture of substances, obtained by the last evaporation of brain-extracts; is, or contains, Couerbe's éléencéphole.

55. **Oleate and Margarate of Ethyle.*—Products of decomposition and combination, probably. Lecithine seems to decompose easily, and its acids, in the status nascens, in the presence of alcohol become ethers. They settle as an oily matter from alcoholic solution, from which all matters precipitable by lead acetate, cadmic chloride, and platinic chloride have been removed. This seems to be Couerbe's éléencéphole in its purest state, and Frémy's "oleine nearly pure." Its bearing on distillation, above 360° C., is quite different from that of either oleine or oleic acid.

56. *Oleic Acid.*—Product of chemolysis of phosphorised substances, notably of lecithine; obtained always together with margaric acid, the latter in largest quantity on the whole. First obtained by Gobley from brain-matters.

57. *Oleine* (Frémy). — The statement of this author will be criticised in the survey; his analyses do not support his assumption. Gobley and myself never found oleine, declared by Frémy to represent the éléencéphol of Couerbe; therefore, possibly, Frémy's oleine is mainly oleate of ethyle, a product of the reaction between decomposing lecithine and the alcohol used for extraction.

58. †*Oleophosphoric Acid* (Frémy). — From the insolubility of the acid in alcohol it might be supposed that the substance was mainly composed of bodies of the kephaline series. But these contain all nitrogen; Frémy's body is said to have been free from nitrogen. No inquirer after Frémy has ever found this oleophosphoric acid, and Frémy himself avows that he never had it pure. He never analysed it, and therefore may have overlooked the nitrogen. On the whole I coincide with Gobley's criticism of this acid, and, after many vain endeavours, abandon any further attempts at its interpretation.

59. †*Osmazome* (Vauquelin). — The last water-extract, containing all substances soluble in water, and having while hot a most agreeable odour of broth, which is much finer than that of the purest meat extract.

60. **Oxykephaline.*—A substance of the kephaline group, containing an atom of oxygen more than kephaline, and being more decided in its combining tendencies. Kephaline being somewhat oxydisable under the influence of oxygen, this body might be a product; but this is not proved, and the substance must therefore stand as an educt.

61. *Palmitic Acid.*—Supposed ingredient of margaric acid. See however, the note on this latter acid.

62. *Palmitine.*—If Frémy's derivation of Gobley's oleic acid from oleophosphoric acid (should be oleinophosphoric acid, because supposed to contain oleine in combination with phosphoric acid), were correct, then, says Gobley, he would be compelled to derive his margaric (palmitic and stearic) acid from some margarine (palmitine and stearine), which must be supposed to be decomposed by his process. But solid neutral fats have never been found in brain, and therefore the presence of palmitine is highly improbable.

63. **Paramyeline.*—A phosphorised body, similar to myeline, and also to lecithine, but differing in particulars from both.

64. **Peroxykephaline.*—A phosphorised substance of the kephaline group, containing two atoms of oxygen more than kephaline, and one more than oxykephaline; the most precise body of the group as regards its combining power.

65. *Phosphates* of potassium, calcium, magnesium, and perhaps other metals.

66. *Phosphorus.*—Early brain researches contain evidence of many inquiries after the form in which phosphorus is present in the brain, particularly search after "phosphorus as such," or P in the metalloid form was made. But it is now almost certain that the phosphorised organic matters all contain P in the form of phosphoric acid combined with glycerine, and yield this nucleus by chemolysis as glycerophosphoric acid. It is, however, possible that *some* phosphorised matters contain P in two forms, of which the second, not yet fully known in particular, is not yet proved to appear as glycerophosphoric acid by chemolysis, but remains attached to one of the fatty acid radicals of the combination as kephalophosphoric acid.

67. **Phrenosine.*—A nitrogenised substance of the cerebrine type, containing only half as much nitrogen as cerebrine. Forms substitution products with Br and NO_2.

68. †*Protagon.* (O. Liebreich).—A mixture of much cerebrine phrenosine, kerasine, &c. with myeline and some kephaline, also some holesterine. Was, therefore, mainly Couerbe's cérébrote, Frémy's cerebric acid, but contained a little more myeline than these preparations, which raised its phosphorus a little higher. The neurine which it yielded by chemolysis came from the myeline; the supposed new fatty acid ("vibrating mass"), a mixture of several fatty acids also; the sugar-like body, which reduced copper solution, came from the bodies of the cerebrine group. Its hydrogen was found so high, that it cannot be disposed off by any easy hypothesis.

69. **Pyrokephole.*—Distillation product of (perhaps impure), oleate of ethyle; on analysis gives figures which show it to be empirically isomeric with oleic acid. Perhaps a mixture. According to Laurent, oleate of ethyle distils unchanged; according to others it decomposes completely. If decomposed, it is so in this case without much destruction, that is to say, it is rather permanently dissociated by the effect of heat (thermolysed), than burned at the expense of its oxygen.

70. *Silica.*—Found by some older authors; but uncertain whether derived from glass and porcelain vessels or glace of earthenware.

71. *Stearic Acid* (Diakonow).—Said to have been obtained by chemolysis of lecithine, which this author describes as containing two nuclei of stearyle. Not found and doubted by Strecker.

72. *Stearine.*—To this substance the remarks on palmitine and margarine apply.

73. *Stearoconote* (Couerbe).—A body obtained in small quantities with and from the cerebrine group of bodies; it is insoluble in boiling alcohol and soluble in cold benzole. Can be artificially produced from cerebrine bodies, which I then term "stearoconotised," and retransformed into cerebrine. Erroneously declared by Frémy to be albumen. Müller's resinous drops. Compounds of cerebrines with bases and salts also imitate the features of stearoconote.

74. *Sugar.*—Diabetic sugar in cases of death from diabetes mellitus.

75. *Sulphates and Sulphur.*—The former as inorganic salts, the latter in organic combination in albuminous matters; as physiolytic product, probably in slow decomposition of the brain, in the form of hydrothion, q. v. The finding by Couerbe of sulphur in his phosphorised bodies is no doubt an error caused either by impure reagents (nitric acid) or by decomposition. The normal phosphorised and nitrogenised brain-matters (albumen excepted) contain no sulphur.

76. *Syntonine.*—Supposed to be contained in the insoluble albuminous residue of brain. Not proved.

77. *Trimethylamine.*—Found by me in decomposing brains; perhaps derived from decomposing neurine or choline (of which Mauthner showed that it yielded trimethylamine when kept for some time in the presence of putrescent matter).

78. *Urea.*—Probably always present in small quantities; accumulates in diseases in the brain to such an extent that the cerebro-spinal fluid, in cholera for example, contains as much as two per cent. of urea, that is to say as much as ordinary urine. *See* Report, 1866, p. .

79. *Uric Acid.*—(Müller), in ox brain, not in that of man. But the identity is not well proved.

80. *Water.*—Nearly four-fifths of the weight of the brain consist of water.

81. *White Matter* (Vauquelin). The total deposit from first hot alcohol extract of brain deposited on cooling. A convenient designation of a bulky crude product containing the ingredients enumerated elsewhere, mainly the group of the cerebrines, kephalines, myelines, and cholesterine. This is sometimes erroneously identified with Couerbe's cerebrote and other substances already more simple.

82. *Xanthine.*—A substance like this body occurs in brain; but its nature is not certainly established.

IV. *Historical Account of previous Researches.*

LIST OF AUTHORS AND DATES OF THEIR PUBLICATIONS.

Hensing, 1715.
Spielmann, 1766.
Mönch, Gurman, Burrhus.
Thouret, 1790.
Fourcroy, 1793.
Vauquelin, 1811.
John, 1814.
Gmelin, 1826.
Kühn, 1828.
Lassaigne, 1830.
Couerbe, 1834.
Frémy, 1841.
Gobley, 1846–1856.
Müller, 1857–1858.
Liebreich, 1864.
Koehler, 1867–1868.
Otto, 1867.
Diakonow, 1867–1868.
Strecker, 1869.
Baeyer, 1868.
Klauss and Kessé, 1867.

Hensing finds phosphorus, 1715.

The earliest distinctly chemical fact ascertained by research conducted on brain matter seems to have been the presence in it of phosphorus. This was discovered by Hensing, and described by him in a dissertation (Examen chemic. cerebri. Giessen, 1715). The discovery was no doubt made by the methods of Brandt and Kunkel, the discoverers of phosphorus, and was one of the many results of the great impulse which the then marvellous productions of these accomplished apothecaries had given to the study of chemistry in the principal European countries. Later in the same century we find Spielmann (Mister. chemiæ Arg. 1766, p. 204) examining the ash of the brain, and discovering in it particles which were attracted by the magnet. Towards the end of the century destructive distillation, which had been for a long period almost the only chemical operation applied to organic, particularly animal, matters, was less frequently used. It had led to the discovery of phosphorus, and of the universal presence of mineral and salty particles in organized beings and all their hard and soft parts and fluids, particularly to the discovery of iron as an essential constituent of the red particles of the blood (Menghini). But its powers for discovery were long exhausted, and other reagents, particularly of the class of the acids, were applied to organic, and amongst them brain matters. Thus we learn from Soemmering's Hirnlehre, p. 81, that Mönch applied nitric acid to brain marrow, and obtained, as a product of its action, oxalic acid.

Spielmann analyses ash, 1766.

Mönch obtains oxalic by nitric acid from brains

(See for above quotations the notes of John to the translation of Vauquelin's research (to be cited below) in Schweigger, 8. (1813) 431).

Gurman; conservation of brain in corpses.

Vauquelin, who gave a history of the chemical labours which had before him been directed upon the brain, was not acquainted with any of the authors just quoted, and gives, as the earliest observation concerning chemical features of the brain, that of Gurman, relating to the prolonged conservation of brain-matter in the skulls of corpses.

Burrhus compares brain to an oil and to spermaceti

A more detailed appreciation of the chemical peculiarities of the brain appears already in the note of Burrhus, also quoted by Vauquelin, who had compared its ingredients to some oil and to spermaceti.

Thouret, 1790.

The great rise of chemistry in France towards the end of the last century inspired the celebrated physician Thouret, the contemporary and friend of Lavoisier and of Vauquelin, with the desire to institute some chemical researches on the human body, and he was led to investigate the brain by the curiosity which at that time existed in Paris, by the observation that of the remains of dead bodies which had been exhumed from the cemetery of the Saints Innocents, many parts, particularly the brains, were in a remarkable state of preservation, although the bodies had been buried so many years, that under ordinary conditions they would have been completely disintegrated. While other inquirers applied themselves to the substance which then received the name of "adipocire," Thouret (Journ. de Phys. 38 (1790) 329) made some inquiries on the brains which led him to the conclusion that they consisted of *a soap*, of which a *fatty*

matter, similar to, if not identical with, spermaceti, and a *fixed alkali*, were the proximate constituents.

Supposed spermaceti.

About this time Fourcroy (Ann. de Chim. 16 (1793) 282) instituted some researches on the brains of calves, sheep, and of man. He showed that the brain contained, as chief ingredients, an *albuminous* and a peculiar *animal* matter, and a small quantity of *mineral* salts, namely, the *phosphates of ammonia, soda, and lime*, but no fixed alkali united with fatty bodies after the manner of soaps, as Thouret had stated (regarding the brains from the cemetery, which do not appear to be fair objects of a comparison in which identity of material is assumed as the basis of the criticism. Th.). Fourcroy subjected brain matter to the action of heat, of water, of sulphuric acid, of dilute nitric acid, and of alcohol. With boiling alcohol he extracted a matter which, on cooling of the solution, was deposited in brilliant plates of a yellowish white colour. This was probably the same substance as that which Burrhus and Thouret had considered analogous to spermaceti, and which, from the description, we easily recognise as the substance now termed cholesterine. Fourcroy showed its differences from spermaceti, and declared its peculiarity, but without giving it any name. It is singular that Fourcroy, though the author of these experiments, which Vauquelin praises as the most complete and successful ever instituted before his own, gave no account whatever of the chemical properties of brain substance in the fourth volume of his work on chemistry, which was published some years after the publication of his essay. (I refer to the English translation, in 4 vols., by R. Heron. London, 1796.)

Fourcroy, 1793.

Mineral phosphates.

Alcohol first employed.

Crystals are not spermaceti.

Vauquelin, 1811.

In 1811, Vauquelin (Ann. du Mus. d'Hist. Nat., 1811, p. 212–239: also in Ann. Chim., 81, 37: English translation in Ann. of Philos. 1, 332; German in Schweigger, 8 (1813) 430–460) published a remarkable research on the brain and nerve substance of man and animals.

In order to appreciate fully the great value of this inquiry, we must consider somewhat in detail the proceedings and results of the great animal chemist.

Vauquelin's process.

Human brain was pounded and triturated in a mortar until it presented a homogeneous paste; it was then mixed with five parts of alcohol of 36 degrees, allowed to stand 24 hours, and then heated to ebullition and filtered. The alcohol became greenish, and deposited, on cooling, a white matter, partly in flakes, partly in scales. After standing 12 hours, the alcohol was filtered from the deposit; it had retained its greenish colour, and became milky when mixed with water. This alcohol was next evaporated to one-eighth of its original bulk; it deposited during cooling an oily yellowish and fluid matter, which sank to the bottom of the vessel containing the now yellow fluid. This fluid resembled a solution of gum. The extraction of the brain matter with alcohol was repeated, and the extracts were treated like the first one. The solution had a more bluish colour. (This green colour, it may be at once stated, is partly due to a fluorescent ingredient of the brain, partly to copper, either contained in the brain or imported as an impurity; but the blue colour Vauquelin extracted from his filtering paper. Such is the opinion of Couerbe, and I have myself been obliged to abandon the use, in my brain researches, of the otherwise excellent grey French hand-made filtering paper, because the solution of brain-matter in alcohol extracted from it a blue or violet colouring matter.)

White matter in scales and flakes.

Colour of alcohol explained.

White matter.

Now, although Vauquelin had observed that the first deposit from the hot alcohol extract consisted of a *flocculent* and a *scaly* matter, therefore of two matters differing in their physical appearances, yet this does not appear to have made the necessary impression upon him. He made no attempt to separate these matters, either mechanically or by solvents, but treated the entire deposit as a homogeneous substance. He described it correctly as white and concrete, yet soft and plastic, shining like white silk, and staining paper like grease. He found it fusible by heat, but never so liquid as fat, and assuming a brown colour at a temperature at which fat is not changed. It dissolved in hot alcohol, but left flakes of an *animal matter* undissolved, which he believes to have been held in solution only with the aid of the water primarily contained in the brain. Like all subsequent observers, except Couerbe, he threw this matter aside (Couerbe's cephalote and stearoconote) as if it were an albuminous impurity, and thus missed, like all observers after Couerbe, these most important ingredients. He determined, however, that 20 parts of alcohol of 36 degrees were sufficient to dissolve all that is soluble in one part of white

Stearoconote missed.

matter. Exposed to the sun, the white matter became yellow, a phenomenon which John explains as the effect of incipient oxydation. After repeated digestion in alcohol to separate the last traces of animal matter (stearoconote), a portion was burned in a platinum dish, and left a charcoal containing an acid which precipitated lime-water. He explained this correctly as evidence of the presence of phosphoric acid, which he thought might be contained in the white matter as such, or combined with ammonia, losing its base by heat, though he thought this surmise improbable.

Phosphoric acid in white matter.

Desirous of extracting any phosphate with water, he triturated some white matter with water, and was surprised to find that it formed a sort of emulsion, and remained quite neutral to litmus. Potash evolved no ammonia, even on boiling, and even a quantity of potash which would have dissolved any similar amount of fat, did not effect a solution, and the liquid remained as milky as if water alone had been employed. Thus it was proved that the white matter contained neither phosphoric acid nor ammonic phosphate.

Fusion with potash.

One part of white matter heated with two parts of caustic potash and a little water, did not fuse; on the contrary, it became hard, which could not have occurred if the matter had been fat. After evaporation of the water, the mixture assumed a brown colour, took flame, and exhaled an odour of burned fat, with much soot. The residue was extracted with water, the extract boiled with nitric acid, and the solution then yielded to lime water, calcium phosphate, which when dry weighed one-tenth of the white matter employed. One part of white matter, deflagrated gradually in two parts of fused nitre, yielded by appropriate treatment as much calcium phosphate as the previous operation.

Deflagration with nitre.

Phosphorus in a peculiar form.

From these phenomena Vauquelin concludes that with the white fatty matter of the brain, there is combined a quantity of *phosphorus*, which dissolves in alcohol at the same time as the matter itself. The matter contains neither lime nor magnesia, though they are contained as acid phosphates in the alcoholic mother liquor, together with phosphate of potassium. From these phenomena Vauquelin concludes that in the brain phosphorus is present in the same form as that which he said Fourcroy discovered in the roe of fishes. Judging from the quantity of phosphate of lime obtained, he believes the quantity of phosphorus *to be but small*, and to amount to not above* fifteen thousandths of the wet brain, or to about $7\frac{1}{2}$ per cent. of the dry brain matter.

White matter, a peculiar and new kind of fat.

Vauquelin further says that although this white matter approaches more to the nature of fats than to that of other substances, yet it must not be confounded with ordinary fats. It differs from them by its solubility in alcohol, its crystallisability, its viscosity, its lesser degree of fusibility, and by the black colour which it assumes on fusion. If, therefore, it is desired to range this matter under the class of fats, it must be considered as a peculiar and new kind of fat. Vauquelin evaporated the alcoholic solution from which the white matter had been deposited, in one operation (without, as will be seen to have been done in my experiments, effecting a fractional evaporation, and obtaining one or more intermediate precipitates). Towards the end of the evaporation he observed the formation at the bottom of the fluid of an oily, fluid, yellow matter, which he supposed to result from the "brain moisture." The liquid in the depth of which this oily matter collected, had also a yellow colour, a faintly sweet taste of broth, and showed traces of acidity. As long as the liquid was hot, the matter remained separate, and of a certain consistency; but when the fluid cooled, or on addition of a little cold water, the matter absorbed moisture, lost its translucency, and became diffused in minute flakes so that it could not be separated any longer; it was, therefore, necessary to observe the favourable moment for its separation, and to employ hot water for washing it and separating it from the soluble admixtures. The oily matter was dried at a gentle heat, or in the open air; it was brownish red, had an odour like that, or stronger than that, of brain, and a rancid taste; triturated with cold water it diffused in it, and formed a kind of uniform emulsion, which separated only very slowly. Mineral acids mixed with this emulsion, separated immediately the oily matter in form of white opaque flakes, and the fluid now filtered clear, which it had not done before. From the hydrochloric acid which had been employed to coagulate this emulsion, ammonia afterwards precipitated white light flakes. But when nitric acid had been employed for the same

Oily deposit.

Hot water required to wash and collect it.

Reactions of oily matter.

* Here is a misprint saying $\frac{5}{100}$, which must be wrong from the context.

purpose, neither ammonia nor lime water produced any precipitate in it. The emulsion was also coagulated by tincture of galls.

Oily matter putrifies.

The oily matter, from which the water had been decanted, putrified, and spread a stinking odour.

With alcohol.

The oily matter was soluble in hot alcohol, a few flakes excepted, which did not amount to one per cent., the greater part was deposited again on cooling; water made the alcohol solution turbid like that of a resin. Exposed to glowing heat the oily matter fused, became black, frothed, and exhaled the odour at first of albuminous, afterwards of fatty burning matter. Burned with potash or nitre, it gave phosphoric acid, like the white matter. It contained neither free phosphoric acid nor phosphates. From 400 grammes of brain matter, Vauquelin obtained three grains of this matter, equal to 0·75 per cent. The oily matter differed from the white matter by its red-brown colour, its lesser consistency, its broth like taste, and a greater tendency to crystallisation. For when hot alcohol was saturated with it it deposited scales or crystals on cooling. Nevertheless, Vauquelin concludes that the oily matter is in the main the same as the white matter, but mixed with a certain amount of animal (albuminous) matter, which could be separated from the fatty part by cold alcohol.

Burned, gave phosphoric acid.

Peculiarities of the oily matter.

Yellow watery mother liquor.

The yellow watery mother liquor which remained when the white and oily matter had been deposited, and all the alcohol evaporated, had an acid reaction, and a sugary and broth-like taste. It was diluted with water, and then treated with lime water, until this reagent produced no longer any precipitate. The precipitate was washed, dried, calcined, dissolved in nitric acid, precipitated by ammonia, and was found to be pure phosphate of lime.

Phosphoric acid removed.

Solution evaporated to dryness.

The filtrate from the lime water precipitate was evaporated to dryness, the residue was reddish brown, semi-transparent, had the same somewhat sweet and gravy-like taste as before, and dissolved in alcohol with facility, leaving some saline matter which effervesced with acids. Exposed to the air it deliquesced; burned in a platinum crucible, it emitted vapours of burning animal matter, with much frothing; it left a charcoal, which by extraction with water and evaporation yielded pure carbonate of potassium. From these reactions Vauquelin concluded that the matter in question was identical with that which Rouelle had formerly termed "soap-like extract of flesh," and to which Thénard had given the name of "osmazome." The brain matter, deprived of its fatty matters, contained albumen and coagulated globules of a membranous substance, the neurilemma. This substance when dry assumed a grey colour, a semi-transparence, and a fracture similar to that of gum-arabic. When put into water it became opaque, swelled up, and softened, and a small part dissolved. In this softened state it dissolved easily in caustic potash by the assistance of heat, and during the solution no ammonia was disengaged. (Difference from caseine of milk.) The potash solution was slightly yellow, and had a weak smell. Acids produced in it a white flocculent precipitate, and disengaged a very fetid odour. Acetate of lead dropped into the potash solution produced a dark brown precipitate, showing the presence of sulphur. (The matters extracted by alcohol from the brain did not contain any sulphur.) By destructive distillation the albumen of the brain yielded carbonate of ammonia in crystals, and a red oil similar to that yielded by common albumen under similar circumstances. By complete combustion it left phosphate of lime and magnesia, but no free phosphoric acid; by deflagration with nitre some sulphuric acid was obtained, confirming the lead and potash test above alluded to. Consequently this matter was identical with albumen, an opinion already enunciated by Fourcroy in the essay above quoted.

Osmazome.

Albumen and neurilemma.

Contains sulphur.

Vauquelin summarised his results as follows:

The brain is composed of—

1. Two fatty matters, which are perhaps identical.
2. Albumen (probably semi-coagulated, and not properly soluble in water).
3. Osmazome.
4. Different salts, amongst them phosphate of lime, phosphate of potash, phosphate of magnesia, and a little common salt.
5. Sulphur.
6. Phosphorus.

He estimated the quantities, as far as such matters could then be estimated, follows :—

1. Water, about - - -	80
2. White fatty matter - -	4·53
3. Red fatty matter - - -	0·70
4. Albumen - - - -	7·00
5. Osmazome - - -	1·12
6. Phosphorus - - -	1·50
7. Acid, salts, and sulphur - -	5·15
	100·00

Putrefaction of brain substance.

In the 10th paragraph of his research Vauquelin gives an account of some experiments on the putrefaction of brain matter in the presence of water. It was found that the albumen decomposed rapidly, but the fatty bodies remained almost entirely unchanged; the osmazome also did not decompose. Ammonia and acetic acid were observed as decomposition products, also some valerianic acid, recognized by the smell of old cheese. During putrefaction the mixture maintained a rose colour for several weeks. As the method of physiolysis, so ably used by Proust for splitting up complex substances into simpler factors, was not sufficiently developed at the time of Vauquelin's brain research he did not obtain results of lasting value in this experiment. Vauquelin also examined the *medulla oblongata* and *spinalis*, and found them analogous in composition to the brain, but they contained much more fatty matter, and less albumen, osmazome, and water. This he supposes to be the reason why the spinal marrow has greater consistence than the brain.

Medulla oblongata and spinalis.

Nerves.

Vauquelin found the nerves of the same nature as the brain, but containing much less fatty matter and much more albumen and neurilemma. They contained moreover common fat, which separated from them when they were treated with boiling alcohol. When the nerves were deprived as much as possible of fatty matter by alcohol they became semi-transparent. When digested in that state in boiling water they did not dissolve, but became white, opaque, and swelled up obviously in consequence of absorbing moisture. The residue of nerve which had been treated with alcohol and water dissolved almost completely in caustic potash. No ammonia was evolved during solution. The potash solution was precipitated in purple flocks by acids, and the supernatant fluid also assumed a purple colour.

Philosophic considerations of Vauquelin.

At the conclusion of his paper Vauquelin asks the question whether it might not be possible to recognise the conditions in which each of the elements composing the brain is present in that organ. At first he questioned whether the albumen was not combined with a portion of the phosphoric acid, and whether its consistence and opacity was not perhaps caused by this combination. However that might be, he is inclined to believe that the albumen is in a state of semi-coagulation, after the manner of caseine in milk becoming sour. He next inquires whether fatty matter, albumen, and osmazome are in some combination with each other in the brain, and is inclined to answer the question affirmatively, at least as regards fatty matters and albumen.

Vauquelin's data are perfectly correct as far as they go; they furnished an excellent basis upon which the more perfect methods of later times might have built a complete analysis of brain matter. But the later analysts were all deficient in that appreciation of the totality of the subject which alone gives power for exhaustive research. With the one remarkable exception of Couerbe they were men of detail, fishing for some chemical object of passing interest and neglecting their predecessors, as well as the physiological philosophy of the important organ upon which they undertook to operate.

John's inquiries,

The analyses of Vauquelin were repeated and confirmed by J. F. John (Chemische Schriften, Vol. 4, nro. 31, p. 228; and Zoo-chemische Tabellen, Tab. 1, A (1814), p. 12); also in Gilbert, Annal. 46 (1814), 329), who examined the cerebral matter of calves, deer, chickens, and crayfish. The observation of phosphorus in the human brain caused him to institute a special research on the brains of calves and oxen, which led him to the conclusion, as he expressed it, "that they did not contain any substantial phosphorus." He meant, no doubt, phosphorus in the metalloid form, as it is met with at the point of

a lucifer match, and as distinguished from the phosphorus in combination, such as is known to be contained in bones, as phosphate of lime. He found phosphate of ammonia in his extracts, or rather phosphoric acid in one experiment, and ammonia in another, and therefore believed that the phosphoric acid found by Vauquelin was the result of a decomposition of this ammonia phosphate; he thus assumed against Vauquelin the correctness of a surmise which Vauquelin had at first formed himself, but had ultimately dismissed as very improbable. The researches of John are by no means so clear and instructive as those of Vauquelin, though made by the aid of and after them; they contain not a single new fact or development, their direction is throughout governed and consequently confined by the search after the supposed "substantial phophorus," and they consequently miss entirely the fact that in the brain phosphorus is present in *organic combination*.

L. Gmelin diagnoses cholesterine and pulverulent brain fat.

A real progress was effected by the research of Leopold Gmelin (Report f. d. Pharm. 52, 169), and in Zeitschr. f. d. Physiol. von Tiedemann, und Treviranus, 1, 119; also in Gmelin and Tiedemann die Verdauung nach Versuchen, 1826), in consequence of which he distinguished a *pulverulent brain fat*, and a scaly crystalline one, which latter he identified with the *cholesterine* of gall stones. The latter he obtained by boiling brain with alcohol, crystallising the extract, and recrystallising the product with frequent pressing between bibulous paper, until it was free from smeary and tallow-like matter, and represented small crystalline plates. The wax-like brain fat he found to adhere to the bottom of the vessel in which cholesterine crystallised and separated it mechanically. These important results were not equalled by O. B. Kühn's researches (De cholesterino eique similibus pinguedinis corporis humani formis, Diss. Lipsiæ, 1828); (Extract in Kastner's Arch. f. Naturlehre, 13 (1828), 337), which had a retrograde effect. He separated cholesterine from Vauquelin's white matter by ether, and obtained it mixed with phosphorized bodies, but believing his product to be a new body and neglecting to apply Gmelin's process of purification, or unsuccessful in its application, he termed it *cerebrin*, distinguished it from the cholesterine of gall stones and controverted Gmelin, as we now know, erroneously. Kühn also obtained the pulverulent brain fat as the matter insoluble or least soluble in ether, and termed it *myelokon* (marrow powder). He also seems to have first noticed the brown matter which was afterwards described by Couerbe as céphalote.

Kühn's researches: myelokon and cerebrine.

Lassaigne 1830, on retina and optic nerves.

In 1830 Lassaigne (Ann. Chim. 45, 215; see also Journ. de Chim. Méd. 2nde serie, 1, 344; Compt. Rend. 9, 703; 11, 763) gave a chemical analysis of the retina and optic nerves. He found their constituents identical in kind, but the retina contained more water than the optic nerve.

The constituents of the retina were in 100 parts :—

Water - - - -	92·90
Saponifiable fat and cerebrin - -	0·85
Albumen - - - -	6·25

The optic nerve yielded in 100 parts :—

Water - - - -	70·36
Cerebrin - - - -	4·40
Osmazome and common salt - -	0·42
Gelatine - - - -	2·75

Couerbe 34.

The most extensive and after that of Vauquelin most fruitful research on the chemistry of the brain was published in 1834 by J. P. Couerbe. It was presented by him to the Academy of Sciences of Paris, on June 30, 1834; but meeting with little favour from that body it was published in Ann. Chim. 2nde serie, 56, 160–193. Couerbe experienced the strongest critical opposition on the part of Frémy, as we shall see below, where it will also appear what a great amount of truth there was in Couerbe's work, and how little in that of his opponent. After the publication of Frémy's own research Couerbe again endeavoured to urge his claim to consideration (Nouvelles considerations sur le cerveau. Compt. Rend. 10 (1840), 974), and defended himself against the criticisms of the apparently weightier chemist. The Academy appointed a committee of reporters, comprising Messrs. Chevreul, Dumas, and Pelouze, and this committee, swayed by a prominent error in Couerbe's statements, an

error which might have easily been eliminated from the mass of prosperous truth, namely, the alleged finding of sulphur as a constituent of brain fats, rejected Couerbe's appeal, on nearly this argument, that as Couerbe's statement of the presence of sulphur had been disproved by Frémy, therefore the whole of his researches were of no value.

Couerbe announced that he had isolated from the brain of man the following immediate principles :—

1. A yellow pulverulent fat = Stéaroconote.
2. A yellow elastic fat = Céphalote.
3. A yellowish red oil = Éléencéphol.
4. The white fatty matter of Vauquelin = Cérébrote.
5. Cholestérine.

For the isolation of these matters he proceeded in the following manner. He washed the brain, freed from its membranes, with cold water, to deprive it as much as possible of blood. He then reduced it to a pulp in a mortar, and macerated it in cold ether. The first portion of ether removed little except water. Four successive macerations were requisite to deprive the brain of everything which the ether was capable of dissolving. The ether was now distilled off, and the residue was dried in a capsule, and was found to be a white fatty substance, partly amorphous, partly granular. Almost the whole of this residue (when the brain came from a healthy person) consisted of *cérébrote*, but when the brain was that of an insane person, the cérébrote was combined (mixed) with some other substances. This residue Couerbe termed "product " of the ether treatment A."

The brain matter thus extracted with and insoluble in ether was treated with boiling alcohol of 40° strength, and filtered hot, and this treatment was repeated until the solutions gave no longer any precipitate on cooling, this being to him the signal that the brain matters were exhausted of all matters soluble in boiling alcohol. The fibrous residue he termed névrilème, and did not investigate any further.

(Several authors, amongst them Thomson--chemistry of animal bodies—omit all notice of this alcohol treatment, and state that in repeating the researches of Couerbe they could not find several of the bodies mentioned by him. This omission may perhaps explain the futility of their experiments, as well as of their criticisms).

Deposits from boiling alcohol.

The alcoholic solutions were cooled, decanted, and filtered, and the deposits were collected. They were washed with cold ether, which removed *cholesterine* and left on the filter *cérébrote*. This latter he declares to have been the main ingredient of Vauquelin's white matter, and points out that as Vauquelin did not employ ether either before extraction of the brain with alcohol or in the purification of the white matter, he must have had cérébrote mixed with a large bulk of cholesterine.

Second deposit from concentrated alcohol extract.

The alcohol in which the white powder had been precipitated was further evaporated, in order to obtain a second deposit of this white matter, which continued to fall down, accompanied, however, by other fats soluble in ether.

Semi-fluid deposits.

Towards the end of the evaporation a semi-fluid matter was seen to deposit, which was no longer the same as the white matter of the previous deposits. It dissolved in ether, and was deposited in an oily state by the evaporation of the ether. This matter, already noticed by Vauquelin, Couerbe termed *éléencéphol*. Thus it appears that, as Couerbe himself stated, his alcohol extraction, following the ether extraction, yielded him the substances obtained by Vauquelin. But Vauquelin erroneously held the oily matter to be identical with the white matter, and differing from it only by being less pure. Couerbe established the peculiarity of this matter, as will appear lower down.

Treatment of the ether extract according to whether more or less cérébrote is present.

The extract A obtained by ether from brain previous to the alcohol treatment was subjected to the following processes. It was, as already stated, evaporated to dryness in a capsule, and then again treated with a *little* ether. This was of uncertain effect, for sometimes the entire residue dissolved again in the ether (in the case, namely, in which the ether had taken up but little cérébrote, and the ether was warmer), and then ether was unavailing to effect any further separation, and was again driven off by heat. Sometimes, however (in the case, perhaps, in which the ether had taken up much cérébrote, or the ether was cold), the operation of treating the residue with little ether left a white

powder, undissolved cérébrote, which was separated by the filter. The solution also was again freed from ether by evaporation, and the anomaly of excess of cérébrote being removed, the ether extract residues were now uniform, and all subjected to the following treatment.

Alcohol treatment of ether extract residues.

They were boiled with alcohol, which dissolved all the cérébrote (as much, namely, as had been dissolved even in the small quantity of ether) and two other fatty bodies (cholesterine and another to be identified), and left undissolved a *solid brown substance* resembling wax. This brown substance is a mixture of two bodies; one, the greater in quantity, is soluble in ether, and remains, on evaporation of the ether, as a fawn-coloured substance which cannot be powdered—*céphalote*. The part which is not dissolved by ether remains as a brown powder—*stéaroconote*.

Céphalote.

Stéaroconote.

Cholesterin.

The (hot) alcoholic solution, containing cérébrote and two other fatty bodies, is filtered (hot) through animal charcoal, and then left to itself. White fatty crystals are deposited, and an additional quantity of them is obtained by concentrating the liquid. These crystals are a mixture of cérébrote and cholesterin. After collection on a linen filter they are separated by ether, which dissolves the cholesterin and leaves the cérebrote in a pure state. The cholesterin is deposited on evaporation of the ether.

Éléencéphol.

When the alcoholic liquid which has deposited the crystals of cérébrote and cholesterin is further evaporated, *a red oily matter* begins to appear, together with further crystals of cérébote and cholesterine. To obtain this oil, termed éléencéphol, in a separate state, the mixture of liquid, oil, and crystals is put into a linen cloth and squeezed; the alcohol with the oil passes through the cloth, while the crystals remain upon the cloth. Couerbe then added to the muddy alcoholic liquid a little ether, which dissolved the oil and rendered the liquid transparent. He then set the solution aside, whereupon the oil gradually subsided, while the crystalline matter remained dissolved in the alcohol and ether mixture. The oil was ultimately separated by filtration.

The cérebrote of Couerbe is sufficiently characterized as the pulverulent brain fat, insoluble in water and in ether, easily soluble in boiling alcohol, and little soluble in cold alcohol, properties upon which its extraction is based. It is infusible, and does not cause greasy spots on paper. It is not saponified by either caustic, soda, or potash. Thus far the description of cérébrote by Couerbe is quite correct. By elementary analysis Couerbe obtained °/₀:

C	67·878
H	11·100
N	3·399
S	2·138
P	2·332
O	13·213

No sulphur had been found by Vauquelin, and of this Couerbe was aware. Frémy and all subsequent observers failed in finding sulphur in these peculiar brain matters, and the reporters of the Academy rejected Couerbe's research upon the ground of this erroneous statement regarding sulphur. The method employed for the separation of the sulphuric acid was that of Berthier, with baryta. The brain matters themselves were not deflagrated with nitre, but boiled with nitric acid. Perhaps the inquirer unfortunately employed impure nitric acid, containing some sulphuric. This appears the more probable, as in all the other analyses (also made with nitric acid) of céphalote, stéaroconote, and éléencéphol he also obtained sulphur, and, what is remarkable, in all nearly the same per-centage.

	Cérébrote.	Céphalote.	Stéaroconote.	Éléencéphol.
Sulphur	2·138	1·959	2·030	same as céphalote.

Frémy's critique of Couerbe.

Frémy, in his memoir quoted below, criticised the observations of Couerbe, and accounted for the sulphur as due to an admixture of albuminous matter. This is entirely inadmissible, as pure albumen does not contain as much sulphur as was here apparently found in a body entirely soluble in hot alcohol. Frémy also stated that the cérébrote, on combustion with nitre, always left him a lime salt, and from this (as will be seen, erroneously) concludes that the body was an acid. He sums up by declaring cérébrote to have been a mixture of cerebric

acid, cerebrate of lime, and albuminous matter, and thus increased the obscurity under which Couerbe, by a singular want of caution regarding his analytical reagents, had left his new and important discovery of cérébrote.

The céphalote of Couerbe is equally well characterised; solid, brownish, insoluble in alcohol and water, soluble in 25 parts of cold ether; becomes soft by heat, but not fluid; cold, it is elastic and viscous; in boiling alcohol soluble only in inappreciable traces. Destroyed by sulphuric and nitric acid, saponified by caustic alkalies, yielding colourless fatty acids. Thus far this is again in the main a correct description. Elementary composition °/₀ =

Characters of céphalote.

C	66·362
H	10·034
N	3·250
P	2·544
S	1·959
O	15·851

The phosphorus in this substance was less variable than in cérébrote, an observation which future developments will fully explain. Of this céphalote Frémy asserts that it was a mixture of cerebrate of lime, or soda, with traces of albumen and oleophosphoric acid. But this is entirely erroneous. The céphalote of Couerbe was a mixture of two immediate principles and of their salts. The impurities were cerebrine, and a little cholesterine; the assumption of the presence in it of albumen is still less permissible than in the case of cérébrote.

The Stéaroconote was a powdery, greasy matter, whence the name (στεαρ, solid fat; κονις, powder, derivation similar to Kühn's meyelokon). It is yellowish, infusible, not soluble in either water, alcohol, or ether, even when they are boiling. Yet it has been originally extracted by ether from brain matter, as Couerbe supposes, by reason of the quantity employed, and the combination with all the other matters. It is easily soluble in essential and fatty oils. It dissolves in nitric acid on boiling, and reappears as a white acid fat, soluble in hot alcohol, and crystallising from it in scales similar to margaric acid. Its elementary composition °/₀ =

Stéaroconote.

C	59·832
H	9·246
N	9·352
P	2·420
S	2·030
O	17·120

We are struck by the lowness of the carbon as compared to cérébrote, and the reverse bearing of the nitrogen, the more so as we shall below see the easy relation in which this matter or its essence stands to the essence of cérébrote. I have obtained this stéaroconote in every operation upon the human brain, with all the characters given above, excepting the colour and precise elementary composition. I must therefore declare as entirely unfounded the assertion of Frémy, that stéaroconote was mainly composed of the albuminous matter of the brain. Equally unfounded is the assertion of Frémy that the éléencéphol was a mixture of oleine, oleophosphoric acid, cerebric acid, and cholesterin. There is no olein whatever present; the oleophosphoric acid of Frémy is, as will be shown, after Gobley, more problematical than any of Couerbe's principles; and though we must admit the éléencephol to have been impure, yet the peculiarity of its essence cannot for one moment be doubted by those who have produced and treated it.

Éléencéphol is liquid, reddish, of a disagreeable taste, soluble in all proportions in ether, essential and fatty oils. In alcohol it is soluble by aid of heat, but much less easily than in ether. Otherwise it has few remarkable properties, and in composition it closely resembles céphalote, so that Couerbe considers it as isomeric with this body. This description is in the main correct, though less complete than that given by Vauquelin.

Eléencéphol.

It must not be forgotten that Couerbe was the first chemist who applied quantitative elementary analysis to the brain matter he had extracted. The methods of analysing were at that time by no means as perfect as at present; though he had Liebig's carbonic acid bulbs, he had to use a less perfect method

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On the Chemical Constitution of the Brain, by Dr. Thudichum.

for the determination of nitrogen (in a vacuum produced by Gay Lussac's pump); while it is just possible, though hardly probable, that he could obtain all the phosphorus present in his substances by mere treatment with nitric acid.

Cholesterine.

Of cholesterine Couerbe stated that, contrary to all previous authors who had stated that this body was a morbid product in the brain, he had found it regularly in healthy brains, and therefore considered it a normal ingredient, which moreover was present in large quantity. He determined correctly its elementary composition, and other properties, after Kühn and Chevreul.

C	84·895
H	12·099
O	3·006 %

The speculative part of Couerbe's paper has far less value than the pragmatic descriptive one. He gives empiric formulæ for his substances, from which he omits sulphur and phosphorus, and enters into a consideration of substances similarly constituted. He concludes his essay with some philosophic considerations, which had more value for the time in which they were written than for the present period, but are still worthy of perusal by all admirers of striving minds.

It is surprising in the extreme that these researches of Couerbe should not have been followed up and enlarged, that on the contrary they should have been controverted, denied, and almost ridiculed.

Thomson, Chem. of Anim. Bodies (1843), p. 271, states that he attempted to extract the various matters described by Couerbe from the human brain, but, with the exception of cérébrote and cholesterine, unsuccessfully. Thénard in his Traité de Chim. also criticised these observations in the spirit of the judgment of the academy. While the physiologists of the first 20 years following their publication were yet conscientious enough to quote them, many of the later ones just mentioned the substances as chimerical, and then substituted their own much less perfect experiments and views.

Frémy's research, 1841.

This was mainly caused by a publication of Frémy's (Ann. Chim. 2, 463; also Journ. de Pharm. 27, 453), which was thus summarised by Thomson, l.c.p. 270—"Frémy confirmed the existence of cérébrote and cholesterine "discovered by Vauquelin and Couerbe, but showed that cérébrote when pure "possesses acid properties, and on that account distinguished it by the name "of *cerebric acid*. He found also in the brain an acid, to which he gave the "name of *oleophosphoric*, which he considers as a compound of oleine and "phosphoric acid. He extracted also oleic and margaric acid from brain, and "agrees with Vauquelin in admitting the presence of a considerable quantity "of albuminous matter."

Frémy's proceeding.

Frémy cut the brain into small pieces, heated it repeatedly with boiling alcohol, and left it for some days in alcohol, in order to withdraw as much of the water as possible. Thus deprived of its elasticity, the brain was subjected to the action of a press. It was then quickly pounded in a mortar, and treated with ether. This operation was effected with the least possible exposure to air, in order to prevent the brain from becoming moist again.

The ether extracts, one cold, the other hot, on distillation left a viscous residue, which Frémy termed "ethereal product." After extraction with ether, the brain matter was boiled with alcohol. The alcoholic decoctions deposited the peculiar matter discovered by Vauquelin, containing phosphorus. After this deposition the alcohol contained only fatty matters in solution, and presented an acid reaction, due to the presence of phosphoric acid.

Frémy thus extracted from the brain:

1. A white matter, which he named cerebric acid, and identified to some extent with the white matter of Vauquelin and the cérébrote of Couerbe.
2. Cholesterine.
3. A particular fatty acid, *oleophosphoric acid.*
4. Traces of oleine, margarine, and fatty acids. Frémy stated that these matters were not always contained in the brain in a free state. Cerebric acid according to him was frequently combined with soda or phosphate of lime. The *oleophosphoric* acid he assumed to be ordinarily in the state of soda salt.

Special proceedings for preparing cerebric acid.

To extract cerebric acid Frémy treated the residue from the ether extractions ("ethereal products") with a great quantity of ether. He precipitated thereby

a white matter, which he isolated by decantation, and which on exposure to air transformed itself into a waxy, greasy mass. This precipitate contained cerebric acid, often combined with phosphate of lime, or with soda; oleophosphoric acid, combined with phosphate of lime or soda; and albumen, carried hither by the impurities. The precipitate was dissolved in boiling absolute alcohol, which had been made slightly acid by sulphuric acid. Sulphates of lime and soda remained suspended, mixed with the albumen, and were removed by filtration. Cerebric and oleophosphoric acids were in solution, and deposited on cooling. The deposit was washed with cold ether, which dissolved the oleophosphoric acid, and left the cerebric acid behind. The cerebric acid was then dissolved in boiling ether, and repeatedly recrystallised.

Properties of cerebric acid.

The cerebric acid thus purified was white, and appeared in the shape of little crystalline granules; it was entirely soluble in boiling alcohol, nearly insoluble in cold ether, more soluble in boiling ether. It had the remarkable property of swelling like starch in hot water, but appeared to be insoluble in this fluid. It fused at higher temperatures and then was easily decomposed. It burned under evolution of a peculiar smell, leaving a quantity of charcoal, which burned with difficulty, and was sensibly acid. Sulphuric acid made it black; nitric acid decomposed it, only very slowly. Burned with nitre and soda carbonate the cerebric acid never yielded potassium sulphate, but always a quantity of phosphate, which could be determined. Thus the body was free from sulphur, as had been stated by Vauquelin. The cerebric acid contained nitrogen, for on being heated with an excess of potassa it evolved ammonia. The elementary analyses of Frémy were made by the ordinary methods. The phosphorus he determined as baryum phosphate avoiding the presence of carbonate, or as iron phosphate, according to the process of Berthier.

The analyses yielded him the following results :—

C	66·7
H	10·6
N	2·3
P	0·9
O	19·5
	100·0

Cerebric acid may be combined with all bases, and must be considered as a true acid. It differs from the ordinary organic acids by its insolubility in water and by its other physical properties. Its solubility in alcohol and boiling ether approach it to the fatty acids, but its high fusing point, and its affinity for water, by which it is hydrated, remove it much from the fatty acids.

Cerebrates.

When cerebric acid is heated with dilute solutions of potash, soda, or ammonia, it does not dissolve, but combines with each of these bases. The compounds may also be obtained by mixing any of these bases with an alcoholic solution of cerebric acid; a precipitate almost insoluble in alcohol, which immediately ensues, must be considered as a compound of cerebric acid and the base employed. Lime, baryta, and strontia, combine directly with cerebric acid, and deprive it of its peculiarity to swell and form emulsion with water.

Cerebrate of baryum was prepared by boiling cerebric acid in water, in order to hydrate it; an excess of baryta water was then thrown into the fluid; the mixture was then heated to boiling for some time, carbonic acid being carefully excluded. A white flaky insoluble precipitate was thus obtained, which dried with ordinary precautions showed the following composition :—

Salt -	0·141
Baryta -	0·011
Cerebric acid	0·130

corresponding to 7·8 per cent. of baryta.

This compound did not enable Frémy to calculate the atomic weight of cerebric acid any more than his elementary analyses of the free acid had enabled him to calculate even an empirical formula.

The analysis was unreliable as being made on so small a quantity that the mere removal of phosphoric acid must have caused the result to be highly inaccurate.

Attempted decomposition of cerebric acid.

When Frémy had recognized that acid solvents decomposed oleophosphoric acid completely into oleine and phosphoric acid, he tried to effect a similar decomposition upon cerebric acid, hoping to split it up into a solid fatty body and phosphoric acid. He foresaw that the decomposition would have to be complicated, on account of the nitrogen present. But his experiments remained unsuccessful, because the cerebric acid was never completely decomposed, and always retained phosphorus.

Oleophosphoric acid.

The ether which had been used for the precipitation of the cerebric acid from the ether product contains in solution a viscous matter, which is in part oleophosphoric acid, often combined with soda. In order to obtain free oleophosphoric acid it is necessary to decompose the salt with an acid, and to dissolve the acid in boiling alcohol, from which on cooling it is reprecipitated The acid can be freed from some oleine which is always admixed by absolute alcohol, and from cholesterin by ether. Nevertheless, Frémy did not succeed in preparing the oleophosphoric acid in a state of entire purity, as it always contained cholesterin and cerebric acid.

Not obtained pure.

Colour.

The oleophosphoric acid is ordinarily coloured yellow like oleine. It is insoluble in water, and swells a little when put in boiling water, a phenomenon which Frémy supposes to arise from the presence of cerebric acid. It has a viscous consistence. It is insoluble in cold alcohol, but dissolves easily in boiling alcohol; it is soluble in ether. When put in contact with potash, soda, or ammonia, it immediately yields soap-like compounds, which have again all the properties of the matter extracted from the brain substance by the first ether treatment. With the other bases it forms insoluble compounds. It burns in air, and leaves a strongly acid charcoal, in which the presence of phosphoric acid can be recognised.

Soluble in, or insoluble in, alcohol, &c.

Salts: alkaline, earthy.

Burns: leaves phosphoric acid.

Oleophosphoric acid is decomposed yielding oleine and phosphoric acid.

The liquid greasy substances obtained from the brain do not always show the same properties. Sometimes they are liquid like oleine, at others they are on the contrary viscous. In come cases they contain phosphorus, in others not any. This arises from the decomposition of the oleophosphoric acid which can be engendered artificially. When it is boiled for a long time in water or alcohol, it gradually loses its viscosity, and is transformed into a fluid oil, oleine, almost pure. The liquid has a strongly acid reaction, due to the newly-formed phosphoric acid.

Decomposition by boiling, particularly in acid fluids.

This decomposition, which is always long and incomplete when oleophosphoric acid is boiled in pure alcohol or water, may be effected very quickly by making the liquid slightly acid. The decomposition also takes place at the ordinary temperature, but is then very slow. The air does not seem to take any part in this change.

The oleophosphoric acid is not a mixture.

Yields oleine by decomposition.

It is possible to prove by several proceedings that the oleophosphoric acid if not a mere mixture of oleine and phosphoric acid. Oleine is soluble in cold absolute alcohol, while oleophosphoric acid is entirely insoluble in it. If then an oleophosphoric acid, which is thus insoluble, is decomposed by boiling with acidified water, it is found to have changed its nature after some time, and that the oily matter produced is now soluble in cold alcohol. It has become oleine, which burns on platinum foil without leaving any carbonaceous residue, while the oleophosphoric acid always leaves a strongly acid charcoal.

Oleophosphoric acid decomposed by putrefaction.

The oleophosphoric acid is decomposed under the influence of putrefying animal matter in the same manner as by boiling with acids. Fresh brains always contain oleophosphoric acid, while brains which had been left for some time, and which began to decompose, yielded much oleine and free phosphoric acid.

No elementary analyses.

Frémy did not publish any analyses of his oleophosphoric acid, as he was convinced that those which he had made had been performed upon impure products.

Easily attacked by fuming nitric acid.

Amount of phosphorus.

The oleophosphoric acid may be easily attacked by fuming nitric acid; this reagent produces phosphoric acid, which remains in solution, and a fatty acid, which floats upon the fluid. Frémy in determining by this process the phosphorus contained in the oleophosphoric acid, found in several analyses, quantities varying between 1·9 and 2·0 %.

Alkalies split up the oleophosphoric acid.

Alkalies in excess transform the oleophosphoric acid into phosphates, oleates, and glycerine. It has not been possible to form artificially oleophosphoric acid, although a compound of sulphuric acid and oleine is known as artificially producible.

Chevreul in an article in the Dictionnaire des Sciences Naturelles had already expressed the opinion that the fatty matter of the brain could be considered as a compound of oleine and phosphoric acid.

The brain often contains an oily matter, which has been found by most chemists studying the brain. This, according to Frémy, came from a spontaneous decomposition of the oleophosphoric acid. Frémy showed how the oleine could be obtained in considerable quantity, by decomposing the oleophosphoric acid by means of acidulated alcohol or water. When the acid thus boiled has lost its syrupy consistence, it is transformed into a fluid oil. This is repeatedly washed and then extracted with cold absolute alcohol. This agent dissolves the oleine, and leaves behind the cerebric acid and the cholesterine, which the oleophosphoric acid had held in solution. On evaporating the alcohol oleine is obtained with the following characters: it is fluid, greasy to the touch, coloured yellow, and burns with a white flame without leaving any ash. On elementary analysis:—0·204 grm. gave 0·590 of CO_2 or 79·5 % of carbon, and 0·220 grm. of H_2O, or 11·9 % of hydrogen, leaving for oxygen 8·6 %. These proportions are the same as those which Chevreul found for the oleine from human fat; but they are incorrect, trioleine $C_{57} H_{104} O_6$

Oleine of the brain.

Mode of obtaining oleine from acid.

Characters of the oleine obtained.

Analysis of oleine.

requiring	C 77·37	and human oleine yielding	C 77·5
	H 11·76		H 11·5
	O 10·87		O 11·0
	100·00		100·0

(Chevreul, Rech. 185 and 244; *see* Gmelin, 17, 87.)

The oleine from the brain is easily saponified by alkalies yielding oleates and glycerine. Before subjecting oleine to analysis it is necessary to wash it with alcohol of 34° containing a little potash, in order to dissolve any traces of oleic and margaric acid which it might retain. Frémy considered only such oleine as pure which burned without leaving any acid charcoal, thus showing that, according to Frémy, it did not contain any oleophosphoric acid, and which could be entirely transformed into soap, which soap was completely soluble in water, thus proving the absence of cholesterin. (Soap dissolve cholesterine.)

Easily saponified.

Tests of the purity of oleine from the brain.

Oleic acid $C_{18} H_{34} O_2$ requires	Trioleine $C_{57} H_{104} O_6$	Frémy found
C - - 76·59	77·37	79·50
H - - 12·06	11·76	11·9
O - - 11·35	10·87	8·6

In Frémy's alleged oleine the carbon is therefore 2·2 % too high, and the oxygen 2 % too low, as compared to trioleine; it is therefore not impossible that he had *oleic acid or oleate of ethyle mixed with some cholesterine* in his hands. A mixture of an atom of oleic acid with an atom of cholesterine would yield C = 80·29; H = 12; O = 7·6 % figures which differ from Frémy's analytical results much less than these results differ from the theory of oleine. T.)

Frémy gives the following procedure for the isolation of cholesterine. The first ether extract is boiled with alcohol which has been made strongly alkaline with potash. By this proceeding cerebrate, oleate, and phosphate of potash, glycerine, and cholesterine are produced. The alcohol on cooling deposits the cerebrate and phosphate of potash together with the cholesterine. The deposit is now treated with cold ether, which dissolves all the cholesterine and that only. It is easily purified by crystallisation.

Mode of obtaining cholesterine.

When the ether product is extracted with alcohol of 34° strength made alkaline with ammonia, an ammoniacal salt is obtained, which on its decomposition yields both oleic and margaric acids. The quantity of these acids in the brain is very small, but they can be well identified in a human brain (?) the margaric acid in particular by its brilliant needles fusing at 60° C. The brain changes quickly and soon putrefies. The oleophosphoric acid is soon decomposed into oleine and phosphoric acid. But other matters must be similarly decomposed, for Frémy always found the fatty acids in greater

Fatty acids obtained from the brain.

Fatty acids increase during putrefaction.

quantity in brains already somewhat decomposed than in fresh ones. He ascribes this decomposing action to the albuminous matter. Frémy puts this experience on a parallel with that of Chevreul made upon the fat of dead bodies. Here also, an albuminous matter split up the fat into glycerin and fatty acids, which latter were in combination either with lime or ammonia.

Parallel with adipocere.

A similar experience has been published regarding palm oil by Pelouze and Felix Boudet. It contains a kind of ferment, which can transform it into glycerin and fatty acids.

Palm oil similarly decomposed.

Fatty acids deposited in the brain in disease.

In disease such decomposition can perhaps become very considerable, or continue for a long time in the brain. Felix Boudet examined a concretion from the brain of a fœtus, and found it to contain margaric acid fusing at 60°.

Albuminous matter.

A certain quantity of albuminous matter passes into the ethereal extract by means of the fatty matters. It is left undissolved by absolute alcohol, mixed with a little acid. It has the characters of albumen, and contains sulphur (probably also stearoconote).

Softening of the brain.

Frémy found that in softening of the brain the fatty matters underwent the same decomposition which the oleophosphoric acid exhibits when it is in contact with a decomposing animal matter out of the body. He has seen, he says, that the modification which brain matter suffered is due to a veritable putrefaction, which, in acting upon the albuminous matter, destroys its consistence and changes it profoundly.

Differences between white and grey matter.

Frémy stated that the greasy matters were almost always entirely contained in the white matter, and that the grey matter contained but traces of them.

When he had extracted all the greasy matters from the white matter he obtained a residue which presented chemical analogies to the grey matter, but he did not state in what these analogies consisted.

The brains of animals.

The brains of animals contain the same substances as the human brain, but in perhaps different proportions. Thus, upon the same amount of fatty matter, the brain of man contained more cholesterin than the brain of a dog.

Brain fats in the blood.

The analyses of the human brain at different ages yielded no positive results.

Chevreul had already found in the blood peculiar fats ordinarily met with in the brain. Cholesterin was found in the blood by Felix Boudet.

Brain fats in other organs.

All organs containing nerves will probably yield brain acids and fats if, as is likely, nerves have the same chemical constitution as the brain. The circumstance that the liver could secrete so much cholesterin caused Frémy to search for brain acids and fats in it, and he succeeded in extracting oleophosphoric and cerebric acids from it.

Gobley's researches on yelks.

The researches of Gobley which were destined ultimately to throw much light on the nature of brain matter, were begun upon the yelks of hens' eggs (Rech. Chimiques sur le Jaune d'Œuf. Journ. de Pharm. et de Chim. 9 (1846), 1, 81, 161. He showed that they contained water and an albuminous matter, which latter he, in accordance with precedent, termed vitelline. He further extracted oleine and margarine, as had been done before, by pressing the coagulated and dried yelks, and by exhausting them with ether; but he found this important difference, that whereas the yelk oil obtained by pressure was a mixture of oleine and margarine purely, which when warm easily filtered through paper, the oil obtained by the intervention of ether did not so filter, but left behind a considerable infusible residue, to which he gave the name of *viscous matter*. This matter remains in the yelk cake when the oil is expressed, and can be extracted by ether afterwards. The quantity of fatty oil obtained from dry yelk was in the mean 21 per cent. It contained only cholesterine and colouring matters, and was free from sulphur and phosphorus. The cholesterine, first discovered in yelks by Lecanu, he further studied and identified by elementary analyses. He also stated that the fusing point of yelk cholesterine, which Lecanu had fixed at 145°, while Chevreul had found biliary cholesterine to fuse at 137°, was not actually different from the latter, and claimed that Chevreul had stated the temperature of the moment of consolidation, whereas Lecanu had given that of fluidification.

Vitelline, oleine and margarine.

Viscous matter.

Cholesterine and its fusing point.

Particulars of viscous matter.

Of the viscous matter, Gobley says that it had been observed by John, who pronounced it to be not of the nature of a fat. It is specially interesting as containing the phosphorus, which remains in the form of phosphoric acid after combustion. It distributes itself in water, and forms with it a kind of emulsion, soluble in ether and alcohol in the cold, but when treated with alcohol leaves a deposit of oleine and margarine. Gobley believed it to be

Components of viscous matter.

constituted of a mixture of oleic acid, margaric acid, and a particular acid containing phosphorus, all combined with ammonia, and forming a true soap; this soap he supposed to be, so to say, surrounded and enveloped by an organic nitrogenised matter, differing from vitelline. He chemolysed the viscous matter by heating it in water, with four times its weight of hydrochloric acid, and obtained an oily matter on the top, an intermediate flaky substance, and a liquid. The oily matter was composed of oleic and margaric acid, with cholesterine and neutral fats in small quantity, free from phosphorus. On solution in ether and filtration it left the greyish flakes on the filter. The acid solution, freed to some extent from hydrochloric acid by evaporation, and then diluted with water, gave a precipitate with neutral lead acetate, which contained all the phosphorus. The filtrate treated with sulphuric acid gave on evaporation a residue which contained ammonium sulphate. By caustic alkalies the same chemolytic products were obtained, the fatty acids being separated by acetic acid. The fatty acids were separated by Gusserow's method, treatment of the lead salts with ether, when oleate dissolved and margarate remained. The purified margaric acid fused at 60°, and contained 75·286 °/₀ C, 12·605 °/₀ H, and 12·109 °/₀ O. The oleic acid gave the commonly accepted quantities of elements. 100 parts of dry yelk yielded by this process in the mean 7·226 parts of fatty acids.

Chemolysis of viscous matter by acids.

Chemolysis of viscous matter by alkalies.

From the viscous matter, decomposed by long boiling with caustic potash, and neutralised with acetic acid, and freed from liberated fatty acids, the phosphorised acid was precipitated by neutral lead acetate. The precipitate was decomposed by hydrothion and the filtrate concentrated. Some hydrochloric acid was removed by silver oxyde, and the excess of the latter again by hydrothion. Some free phosphoric acid was removed by a slight excess of lime water and filtration. The liquid on evaporation, and while hot, deposited white, brilliant scales, which could be removed and dried. They were more soluble in cold than in hot water; the solution was precipitated by alcohol. By analysis the salt yielded :—

Phosphorised acid.

Its lime salt.

	in 100		Theory.
$Ca_2 P_2 O_7$ (calcium pyrophosphate) -	60·27	60·27	60·66
C - - - - -	17·07	17·02	17·06
H - - - - -	3·51	3·48	3·32
O - - - - -	19·15	19·23	18·95

The acid contained in this salt was found to be phospho-glyceric acid, or as it is now most frequently termed, glycero-phosphoric acid, being the acid previously synthetically prepared by Pelouze, and identified all the more certainly as all Gobley's experiments were made in the laboratory of Pelouze.

Gobley further found lactic acid in yelk; then various mineral salts, specially potassic and sodic chloride, potassic sulphate, and calcic and magnesic phosphate. He also distinguished two colouring matters, and meat extract, or osmazome, and concluded his prosperous research by a summary in 12 propositions, embodying the results sketched in the foregoing lines.

Other ingredients.

In a second memoir (Journ. de Pharm. et Chim. 11 (1847), 409, and 12 (1847), 1) Gobley describes his experiments on the brains of chickens, sheep, and men. He obtained, by proceedings which he did not describe, a viscous matter which had much analogy with that obtained from yelks. When shaken with water and little hydrochloric or sulphuric acid, and warmed on the water bath, it did not decompose, while the matter from eggs had thus been decomposed; but when the viscous matter was boiled for some time with dilute acids it became liquified and gradually floated on the fluid. The greasy layer consisted of oleic acid, margaric acid, cholesterine, and a small quantity of fixed oil. The liquid did not contain phosphoric, but *phosphoglyceric acid.*

Gobley's experiments on brain matters.

Viscous matter decomposed by boiling with dilute acids; products.

By digestion with caustic potash the viscous matter could not be transformed into salts of oleic, margaric, and phosphyglyceric acids (the viscous matter from yelks was entirely split up), but by boiling with potash in alcohol these three acids were obtained from the brain matter.

The viscous matter from yelks and brains was soluble in crystallisable acetic acid. It could be boiled with this acid for six hours without showing signs of decomposition. Only after 12 hours' boiling a greasy matter floated on the top, which contained oleic and margaric acid, but contained yet much phosphorus.

Bearing with acetic acid.

It was necessary to boil at least 24 hours to effect a perfect decomposition. The liquid then contained glycerophosphoric acid, but also phosphoric acid and glycerine.

Tartaric and lactic acid acted in the same manner as acetic acid.

Change of opinion: viscous matter a neutral substance.

In this memoir Gobley withdrew his first view of the viscous matter being a mixture of ammonia soaps of the three acids obtained; he removed the soluble salts by washing the boiled yelks with water, extracted and chemolysed by sulphuric acid the viscous matter, and searched for ammonia in his ultimate residues. But the quantity of this base found was so small that it could not be brought into relation to the acids found, and Gobley consequently modified his opinion of the viscous matter in this direction, that it was a neutral matter in which the three acids did not pre-exist, and in fact a complex body from which he had separated two matters, one of which he named *phosphorised matter*, and the other *cerebric matter*. The phosphorized matter he now declared to be identical with Frémy's *oleophosphoric acid*, and the cerebric matter, which he now also isolated from yelks with Frémy's *cerebric acid*. But he combated the theory which this latter chemist had given of oleophosphoric acid, and maintained that the phosphorized matter contained the elements of oleic, margaric, and glycerophosphoric acids, and always split up into those acids, and never into oleine and phosphoric acid, as had been maintained by Frémy. According to Gobley, the fatty matters extracted from brains contain neither potash nor soda, but only phosphate of lime. In this memoir Gobley also replies to objections which had been made to his views of the viscous matter by Sacc (Compt. rend. 22, 649), to the effect that the phosphorus was contained in yelk as phosphorus (hypothesis of John), and only transformed into acid by the drying of the yelk in presence of air.

Opposes Frémy's views of oleophosphoric acid.

Frémy's observations on Gobley's research.

On this memoir of Gobley, Frémy made some characteristic observations (Journ. d. Pharm. et de Chim., 12 (1847) 13,) by way of reclamation for himself. He says that the researches of Gobley taught (1) that the fatty matters of yelk contain brain fat; (2) that in the decomposition of oleophosphoric acid, the glycerophosphoric acid of Pelouze may be formed. He is happy to point out that his (Frémy's) work on the brain has enabled Gobley neatly to characterise the fatty phosphorised substances contained in yelk. These claims were subsequently refuted by Gobley, and with complete justice and success. But following, as they did, as a kind of editorial rider to his publication, they for a time extinguished all sense of appreciation of the new researches in the French scientific public, and nearly prepared for Gobley the same unjust fate as that under the sting of which Couerbe had gone to his grave.

Gobley's researches on the eggs of carps.

In a later memorial, entitled "Chemical Researches on the Eggs of Carps," to which the Academy of Medicine before whom it had been read, gave its approbation (Bul. de l'Acad. de Méd. 15,471; the first two memoirs above sketched had been communicated to the Academy of Sciences), and which was published in the Journ. de Pharm., et de Chim. 17 (1850) 401; and 18 (1850) 107, Gobley stated that he had extracted from these eggs the albuminous matter, to which he gave the name of paravitelline, oleine, and margarine, though in much smaller proportions than from hen's eggs and cholesterine, lecithine, and cerebrine, besides salts, meat extract, and a peculiar acid, an odoriferous and a colouring matter. The eggs were boiled with water and pressed, and then extracted with boiling ether; the ether was evaporated and the residue boiled with alcohol; this on cooling deposited the viscous matter, consisting of cerebrine and lecithine, and in the alcoholic solution floated the crystals of cholesterine. The words *cerebrine* and *lecithine* were pointedly selected to emphasise the difference of these bodies from those described by Frémy as cerebric and oleophosphoric acids. Of all four conceptions lecithine (from eggs) alone has survived the progress of science, and complemented with choline (by Strecker) has become one of the best known bodies of the animal economy.

Lecithine from eggs.

The greater bulk of the deposited viscous matter consisted of lecithine. It was soft, formed an emulsion with water, was little soluble in cold, easily soluble in hot alcohol, and deposited from it on cooling; entirely soluble in ether; not altered by air. Heated it did not fuse, but swelled up, and on combustion left a charcoal soaked with phosphoric acid. Under the influence

of mineral acids and alkalies it always yielded with the greatest facility oleic, margaric, and phospho-glyceric acid.

Gobley next combats the idea of Frémy, that lecithine was a body analogous to his sulpholeic acid, and in fact identical with the oleophosphoric acid spoken of in his brain research. Gobley never obtained oleine and phosphoric acid, but always, oleic, margaric, and glycerophosphoric acid. Thus he says he would be compelled to believe lecithine to be a mixture of oleo-phosphoric and margaro-phosphoric acid. When neutral oleine and margarine are present they are not essential results of the reaction, but admixtures, which were present before the decomposition of lecithine commenced. But how can Frémy explain that he never found margaric acid, which Gobley never missed? Gobley recognized that the peculiar white matter which Vauquelin, Couerbe, and Frémy had extracted from the brain was also present in the viscous matter from eggs of hens and carps. In order to obtain it he decomposed the viscous matter (200 grms.) with alcohol of 88° strength (500 grms.), and 50 grammes of sulphuric or hydrochloric acid. After several hours' standing the mixture was slowly heated to boiling, and then allowed to cool. It deposited white *cerebrin*, with some cholesterine. After filtration the latter was removed by ether. Re-solution in hot alcohol and treatment with ether purified the substance. It is neutral, fuses between 155 and 160°, and becomes black at the same time. In water it swells like starch; it contains no sulphur. It easily retains small quantities of other bases or acids which have been in contact with it, but without combining with them in atomic proportions.

Decomposition products.

Cerebrine from eggs.

By elementary analysis it yielded:

	In 100.	Frémy's cerebric acid.	R. D. Thompson.
C	66·85	66·7	67·04
H	10·82	10·6	10·85
N	2·29	2·3	2·24
P	0·43	0·9	0·46
O	19·61	19·5	19·41

In short the identity of cerebrin from eggs (though it was changed by influence of acid, as shown by fusing point T.) with that from brain seemed proved by this research, although the identity of lecithine from eggs with any of the phosphorised bodies from brain was not yet certainly established.

In the Journ. d. Pharm. et d. Chim. 19 (1851), 406, Gobley published a memoir on the chemical ingredients of the milt of carps, in which he showed that it contained the same ingredients as the eggs. He points out that it presented great analogy in properties and composition with the brain. In vol. 21 (1852), 241, he further describes the fatty matters of the venous blood of man, after having summarised previous researches; he finds oleine, margarine, cholesterine, but maintains the absence of all soaps, or free fatty acids. He further finds lecithine and cerebrine as previously defined by him. The séroline of F. Boudet he considers as a mixture of oleine, margarine, cholesterine, and a little albumen. The blood of the ox yielded the same results as the blood of man. The last memoir of this author, which concerns us in this place, treats of the fatty matters contained in the bile (Journ. d. Pharm. et d. Chim. 30 (1856), 241). He maintains that the fatty acids obtained in the chemolysis or putrefaction of bile are products of the splitting up of lecithine naturally combined in it, but that, although fatty acids are not present in the bile, neutral fats and cholesterine are normally present.

Gobley on the milt of carps.

The same on the fats in the venous blood of man.

Opinion on Boudet's séroline.

The same on the fats in bile.

At this point Gobley relinquished the research; he could not obtain his lecithine in a state of purity, and the methods at his command were evidently exhausted on every field. It was not till Strecker resumed the investigation of lecithine in bile that any progress was made in the recognition of that substance. For Müller, who published his paper in the following year, appears to have completely ignored Gobley's many and laborious researches, and, in some particulars, brilliant discoveries.

Müller published the first part of his researches in Ann. Chem. 103 (1857) 131, of which the following is an abstract:

W. Müller.

Liebig, in his essay on the extract of flesh, had raised the question whether the brain furnished products similar to those of the muscular tissue, or yielded

Liebig's preliminary experiment.

matters peculiar to itself. He pounded the brain substance into a thin milk with baryta water, passed it through a fine hair sieve, and boiled. A coagulum was obtained containing the brain fat, and a clear yellowish liquid, which, after it had been freed from excess of baryta by carbonic acid and heat, contained two baryta salts, one soluble in alcohol, both soluble in water, and giving with acids white flocculent precipitates. Having convinced himself that the extract did not contain any kreatine, he did not carry on the inquiry any farther. It was taken up by Von Bibra, who varied the process a little, by extracting the comminuted brain with cold water, allowing the mixture to stand for 12 hours, decanting the supernatant liquor and coagulating by heat. The clear acid filtrate was treated with baryta water and gently heated, whereby the greater portion of the albuminates was precipitated. The concentrated fluid on standing deposited no crystals, from which Bibra inferred the absence of kreatine; he was not more successful in his endeavour to find inosinic acid, inosite, and hypoxanthine. But he found volatile organic acids, among them formic, identified by the silver reduction test, and fixed organic acids of which he identified lactic.

Two baryta salts obtained, but no kreatine.

Bibra's process.

Fails to find kreatine.

Found formic and lactic acid.

Von Bibra treated the hot alcoholic solution of the substance deposited on the cooling of the alcoholic extract with potash, and boiled, On adding sufficient alcohol, the fluid became of a darker colour, and perfectly clear after some hours. After cooling, and standing 24 hours, nearly all the cerebric acid is deposited as cerebrate of potassium. This is purified by decomposition with hydrochloric acid, &c., until finally pure cerebric acid is obtained. This is a white powder, which is not in the least coloured yellow, it is lighter than water, but swells up in it like starch. It melts at a temperature almost identical with that which effects its decomposition. Bibra gave as the results of his analyses :

Hot alcoholic solution of deposit on cooling of alcoholic extract of brain.

Cerebrate of potassium.

Analysis of cerebric acid.

C	66·93	66·66
H	10·73	10·58
N	2·48	2·54
P	0·52	0·52
O	19·34	19·70
	100·00	100·00

Bibra could not obtain a combination of cerebric acid with either potassium, baryta, copper, or silver, which renders the acid character of this body doubtful. He says that with sulphuric acid in the cold, cerebric acid quickly assumes a purple tint, and after some time becomes black. On treating with an excess of warm dilute sulphuric acid, a black magma is obtained, which, when diluted with water and filtered, leaves on the filter a darkish brown, sticky body, which dries up into a glistening resinous body. With fresh bile, water, and sulphuric acid it assumes a red colour like sugar. With concentrated cold nitric acid after some time, cerebric acid assumes a yellowish tint, and with concentrated hydrochloric acid a feeble violet or reddish tint. Boiled with nitric acid, nitrous acid is evolved, and a clear yellow fluid is finally obtained.

Acid nature doubtful.

Reactions.

If cerebric is boiled with hydrochloric acid, and the boiling be interrupted before everything is dissolved, then on washing the residue with alcohol, and dissolving in boiling alcohol or ether, unchanged cerebric acid (under the microscope) will be found. So that cerebric acid withstands the action of the strong mineral acids pretty long, and is not so easily decomposed as cholesterine.

Not easily attacked by strong acids.

Müller attempted, in the first instance, to repeat and enlarge the experience of Liebig and Bibra.

Human brain was worked up with baryta water to a thin milk, and after 12–18 hours worked through a sieve and boiled, the coagulum was filtered off. The filtrate was yellow, more or less opalescent, and ran through the filter quickly at first, but gradually the filtration proceeded more slowly, and a considerable portion of the fluid remained with the coagulum. A little sulphate of lime solution was added to the fluid before heating as recommended by Liebig, but a clear filtrate could not always be obtained. It was found that by allowing the fluid to boil up strongly many times in succession the

Müller investigates Liebig's two acids.

Method of obtaining a clear filtrate.

coagulum separated in large flakes, whilst the filtrate quickly ran through clear. The yellowish strongly alkaline fluid thus obtained was saturated with carbonic acid. There appeared to be a combination formed between an albumenoid substance and part of the baryta which carbonic acid was unable to split up, as the fluid remained alkaline after passing a strong current of carbonic acid for some hours and subsequent heating.

Filtrate could not be made neutral with carbonic acid.

This filtrate containing Liebig's two baryta salts was evaporated at a gentle heat. Yellowish skins formed on the surface of the fluid during evaporation, and on the sides of the dish a thin yellowish granular matter was deposited, which increased as the liquid became more concentrated. This deposit was filtered off and dried, when it assumed a yellow hornlike appearance, burnt with a nitrogenous smell, and left an ash of carbonate of barium. Its reactions proved it to be an albuminous body.

Filtrate evaporated.

Skins formed.

Deposit is albuminous.

The fluid filtered from this albuminous deposit was evaporated on the water bath until there remained a yellow syrup containing grey membranous flakes. This syrup was now treated with strong alcohol, which threw down a membranous flocculent white precipitate. This precipitate was exhausted with 84 °/₀ spirit. The alcoholic extract should contain the baryta salt soluble in alcohol, and the residue should contain the salt soluble in water. Cold and boiling water, however, extracted but very little substance which was obtained on evaporating the aqueous extract as a yellow mass, burning with a nitrogenous smell and leaving but little ash. It redissolved easily in water, and this solution contained albumen, as shown by the nitric acid and acetic acid, and potassium ferrocyanide tests. The residue insoluble in water proved by the violet colour, which it gave with sulphuric and boiling hydrochloric acid, to belong to the group of albuminous substances.

Filtrate evaporated.

Syrup treated with alcohol.

Residue extracted with water.

Extract contained an albuminous body.

Residue insoluble in water was also albuminous.

The alcoholic solution was yellow, clear, and strongly alkaline. It gave with strong acids and metallic salts white flocculent precipitates, insoluble in excess.

Alcoholic solution was alkaline, precipitated by acids.

By slow evaporation many fine glistening plates were obtained, which by the microscope were shown to be cholesterin. Filtered from these crystals and evaporated a small quantity of a brown smeary fat separated. This was filtered off, and the filtrate after 24 hours standing deposited many square crystals, consisting of sodium chloride and kreatin. The mother liquor contained volatile acids, proved by distillation with sulphuric acid. Other substances could not be distinguished owing to the small quantities in which they were present.

Gave cholesterin, smeary fat, sodium chloride, and kreatin.

Volatile acids.

Other substances.

The above experiment was made with human brains; 25 lbs. of ox brains treated in a similar way gave no better results, and moreover contained no creatin, but furnished a small quantity of a body which from its crystalline form under the microscope seemed to be leucin. The presence of this body appeared to keep the albuminous bodies and fats in a smear so complex that the whole of the material wasted away whilst the author was endeavouring to separate its constituents. It seems from the above that the two acids of Liebig are not constant constituents of the brain.

No creatin from ox brain, but probably leucin.

Liebig's two acids not found.

The author now determined to search for kreatin in larger quantities of brain matter, and at the same time examine all the constituents soluble in water. He employed the method used by Städeler and Frerichs (Verh. der Zürcher Nat. Gesell. IV. 1855), in their researches on the parenchymatous fluids of the tissues, slightly modified.

Lead process for obtaining kreatin.

The brain matter was pounded into a thin milk, with distilled water and acetate of lead solution added, until the whole separated into a clear blood red liquid, and a lower layer of brain pulp; it was then allowed to stand 12–18 hours, well mixed and strained through a fine sieve. The filtrate was boiled, when it separated into a coarse reddish clot, and a clear amber fluid, which filtered readily. If enough lead acetate was not added the separation and filtration were incomplete, but a further addition of lead acetate and heating ensured a successful result.

Brain pounded with lead acetate allowed to stand, heated and filtered.

The filtrate was evaporated to one quarter, and as acetate of lead gave no precipitate basic acetate of lead was added. The white flocculent precipitate thus produced was filtered off, the excess of lead in the filtrate removed by sulphuretted hydrogen, evaporated, the residue dissolved in dilute alcohol, freed from acetic acid by the addition of alcoholic sulphuric acid, the excess of sulphuric acid removed by the careful addition of baryta, and the filtrate

Filtrate evaporated, basic lead acetate added filtered from white precipitate.

Filtrate, SH^2.

App. No 5.

On the Chemical Constitution of the Brain, by Dr. Thudichum.

treated as stated hereafter. 50 lbs. of brains (principally ox) were used; with the exception of kreatin and formic acid all the under-mentioned substances belong to ox brains, whilst these two substances were obtained only from human brains.

Uric Acid.

White precipitate from basic lead acetate treated with H_2SO_4 filtered and filtrate evaporated.

The white precipitate by basic lead acetate was decomposed with sulphuric acid, filtered, and the filtrate evaporated when it became brown. On the surface, a crystalline pellicle formed, the crystals increasing on cooling, and standing for some days, when they were filtered off. They consisted of dark brown grains and plates. Under the microscope part consisted of tables of uric acid, part of dark brown round globules, not changing their form on the addition of acetic acid and warming. They burnt without blackening on platinum foil, melted with the smell of burnt horn, gave off white vapours smelling of cyanogen, and finally volatilised completely without residue. Both bodies were nearly insoluble in water, but easily soluble in potash. The murexide test showed the presence of uric acid; the violet colour, on the addition of potash, vanished rather quickly, leaving a reddish yellow spot, perhaps indicating the presence of xanthin or hypoxanthin.

Inosite.

Filtrate from uric acid crystals gives precipitate of inosite.

The filtrate from the uric acid crystals was evaporated until alcohol gave a permanent precipitate. The liquid was then treated as suggested by Städeler and Clœtta, with an equal volume of alcohol, and warmed slightly, whereby the turbidity vanished. On cooling, and standing several days, many shining cauliflower and stellate transparent crystals were deposited. These were filtered off and recrystallized from water. They consisted of shining rhombic prisms and needles, mostly in rosettes, of a distinctly sweet taste, easily soluble in water, with difficulty in cold strong alcohol, easier in boiling alcohol, separating, on cooling, from the hot alcoholic solution as fine glistening scales; blackening, when heated on platinum foil, with smell of burnt sugar, leaving no residue. It did not reduce a copper solution on boiling. Treated with nitric acid on platinum foil, and carefully evaporated, then treated with calcium chloride and ammonia, and again evaporated, a fleeting but brilliant rose-red colour was produced.

Analysis gave 20 °/₀, or four equivalents of water of crystallisation (16·66°/₀ required)—

Notation in Equivalents.			Found.			
12 C	72	40·00	(by CuO)	39·28	(by Pb Cr O_4)	40·24
12 H	12	6·66		6·77		6·62
12 O	96	53·34		53·95		53·14
		100·00		100·00		100·00

Ten grammes of pure crystallised inosite were obtained from 50 pounds of brain matter used.

Kreatin.

Man, baryta coagulum boiled with 84°/₀ spirit on cooling deposited cholesterin and smeary fat. Na Cl.

The residual portion on the filter from *human* brain after treatment with *baryta*, was exhausted with 84 °/₀ alcohol, and on cooling, a small quantity of cholesterine and a brown smeary fat were deposited. The clear filtrate was allowed to stand for several days, when many crystals formed, partly tetrahedra, partly octohedra, and partly square tables and fine needles, which were filtered off and dried. They were dissolved partially in boiling alcohol, and the solution evaporated, when many regular transparent tetrahedra were obtained, which consisted of sodium chloride, mixed with only a small quantity of organic matter.

Portion insoluble in alcohol was soluble in warm water; consisted of kreatin.

The residue, insoluble in alcohol, was easily soluble in warm water, and was obtained pure by recrystallisation. The crystals had the following properties: colourless shining transparent needles, and flat square tables, odourless, tasteless, without action on vegetable colours; moderately soluble in cold water, easily in warm water, almost insoluble in boiling alcohol. Heated on platinum foil become first opaque from loss of water of crystallisation, then blacken and burn off with smell of burnt horn, leaving no residue. They evolved no trace of ammonia on treating with lime, and were quite inactive to reagents. The solution in hydrochloric or nitric acids quickly evaporated, left fine rhombic prisms. On treating with sulphuric acid, a crystalline residue was left, which under the microscope was seen to consist of very fine four and six sided tables.

From the behaviour of these crystals, they seemed to be kreatin. The estimation of the water of crystallisation and nitrogen confirmed this view.

Contained 12·63 °/₀ of water (lost at 100°) and 32·10 °/₀ nitrogen (by soda-lime, and platinum).

	Theory.		
Atoms.		In 100.	Found.
8 C	48	36·64	—
4 O	32	24·43	—
3 N	42	32·06	32·10
9 H	9	6·87	—
2 Aq	18	12·08	12·63

Kreatin only occurs in very small quantity in the brain, and Müller only proved its presence in human brains; whilst Liebig found 36 grammes in 100 lbs. of the flesh of a lean horse, 30 grammes in 86 lbs. of ox flesh, and 72 grammes in 100 lbs. of lean dog flesh, only half a gramme of kreatin could be obtained from eight human brains, equal to about 24 lbs.

Leucin.

Ox brain, baryta, heat, coagulum extracted with alcohol, crystals apparently of leucin: no kreatin.

Filtrate from basic acetate of lead precipitate; yielded crystals which had the properties of leucin, but not composition.

Lerch, of Prague, also found kreatin in the brain. No kreatin was obtained from ox brain when treated by baryta process, as in the foregoing paragraph, but the alcoholic extract on evaporation gave a crystalline deposit in small quantities, which consisted of leucin. Städeler and Frerichs (Verh. der Zürcher nat. Gesell. iv. 55, S. 14) found leucin in the brain. In the filtrate from the lead precipitate some analogous bodies were found, having the appearance of leucin in ox brain. To extract the leucin the filtrate from the basic lead acetate precipitate was freed from excess of lead by sulphuretted hydrogen, and evaporated to a syrupy consistence, taken up in alcohol, freed from acetate by alcoholic sulphuric acid, the filtrate freed from the excess of sulphuric acid by the careful addition of baryta water, and again evaporated on the water bath to a syrup. The brown residue, smelling strongly of roast meat, was treated with alcohol of 84°/₀, each addition of which produced a transitory turbidity, but after some time the whole of the residue dissolved. Absolute alcohol produced a precipitate of yellowish brown, sticky flakes, which quickly dissolved up in water to a thick brown liquid. The whole was mixed with an equal volume of absolute alcohol, and after standing 24 hours had separated into two layers, a lower blackish brown and an upper clear pale yellow layer. The latter was carefully decanted off, and carefully evaporated on a water bath to a thin syrup. After standing 8 days many yellow granular crystals had separated, and were filtered off. Under the microscope they consisted of large radiate, striped, round, shining globules, very much resembling leucin. Easily soluble in water, with difficulty in cold, but more easily in hot alcohol, separating out from the hot alcoholic solution on cooling as a light, white powder, insoluble in absolute alcohol. It was purified by repeated crystallisations from hot alcohol. Thus purified it appeared as a light powder, tasteless and inodorous, without action on vegetable colours. Heated on platinum foil it blackened and burnt off with the smell of burnt horn, leaving no residue. It sublimed in the usual manner, with the characteristic smell of leucin. It was quite indifferent to reagents. It appeared under the microscope to consist of fine needles concentrically grouped. Burnt with soda lime (and platinum) it gave 13·89°/₀ N. Leucin contains 10·68°/₀. It is not easy to account for this excess of nitrogen, as great care was taken to purify the substance before analysis. Gorup Besanez (Ann. Chem. 98, 15) obtained from the pancreatic glands of oxen a crystalline body very much like leucin, but differing in composition by $C_2 H_2$. The analysis given above would fit with the third homologue of the leucin series $C_8 H_9 N O_4$, which requires 13·58°/₀ of nitrogen. Müller could not find either leucin or any homologue of leucin in human brain. Frerichs and Städeler once detected leucin in the human brain in a case of acute atrophy of the liver, the patient being a female, and leucin abounding in the juices of the other organs. These investigators also searched for leucin in cases of typhus, but with unsatisfactory results. Müller principally investigated brains from cases of tuberculosis and pneumonia, and could never establish the presence of a trace of leucin.

Nitrogen analysis.

Agrees with a homologue of leucin, $C_8 H_9 NO_4$.

Leucin only once found in human brain.

The author never once found leucin in human brain.

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Volatile acids of group $C_n H_{2n} O_2$.

From Bibra's researches it was probable that formic acid existed in the brain. The mother liquor from the kreatin, from the aqueous extract of human brain, was treated with excess of sulphuric acid, whereupon the odour of volatile acids was immediately noticed and distilled. The distillate was colourless, and strongly acid, with a powerful odour of formic or butyric acid. On adding solution of nitrate of silver, a voluminous white precipitate fell, which blackened in the cold, and on boiling was almost entirely reduced. The filtrate remained clear on cooling. On evaporating, a small quantity of fine needles separated, but not enough to determine with certainty the nature of the acid present, but from the appearance of the crystals they were supposed to consist of acetate of silver. Thus in human brain formic acid was found (and possibly acetic).

Mother liquor from kreatin contains formic and possibly acetic acid.

Mother liquor of leucin body from ox brain yields acetic and trace of formic acid.

The aqueous extract of ox brain, after the leucin-like body had been separated, and gave no further precipitate with either alcohol or ether, and was concentrated, treated with dilute sulphuric acid in excess, and distilled. The distillate was colourless, very acid, and smelled strongly of acetic acid; it was neutralised with carbonate of soda, and evaporated; on cooling large rhombic tables of sodium acetate crystallised out, which were separated off. The mother liquor on further concentration left a silky crystalline mass. This was distilled with dilute phosphoric acid. The strongly acid distillate having a powerful odour of acetic acid, reduced a solution of nitrate of silver but slightly. It was neutralised with baryta water and evaporated. On standing it deposited many thin prismatic needles of baryum acetate, containing 59·7% of baryum, pure acetate containing 60·03%. So a trace of formic and a quantity of acetic acid were found in the aqueous extract of ox brain, but all the acetic may have been derived from acetate of lead added.

The acetic acid may have been derived from the lead acetate added.

Lactic acid found by Bibra.

Residue after distilling off volatile acids exhausted with ether.

Ether extract gave calcium lactate.

Lactic acid had been found by Bibra both in the residue after distilling off the volatile acids, and in the fresh aqueous extract obtained according to Liebig's method. Müller's investigation was therefore limited to determining the atomic weight of the calcium salt, and thereby proving the identity. The treatment of the residue was modified, so that if succinic acid were present it might be detected. The residue was therefore first exhausted with ether as long as the ethereal extract continued yellow. The ethereal solution was allowed to evaporate slowly, but the dark yellow, strongly acid syrup which remained did not deposit any crystals, even after standing eight days. The residue was then treated with absolute alcohol, in which it dissolved to a clear yellow fluid. The solution was treated with milk of lime, boiled and filtered hot. On cooling a quantity of white crystals were deposited, partly granular and partly acicular, which under the microscope revealed the well-known tufted forms of calcium lactate. They were purified by recrystallisation from strong alcohol, when they had the following properties:

White, rather light powder, tasteless, inodorous, easily soluble in water and boiling alcohol, but difficultly soluble in cold alcohol. Heated on platinum foil blackens, leaving a residue of calcium carbonate. Gave 29·6% of water, and 18·49% calcium.

	Theory.		In 100.	Found
1 equivalent of	lactic acid	81	= 52·59	—
1 „	lime	28	= 18·18	18·49
5 „	water	45	= 29·23	29·60

12 grm. from 50 lbs. of ox brain.

From 50 lbs of ox brain 12 grammes of calcium lactate were obtained.

Other substances in minute quantities.

Besides the above-mentioned bodies Müller found none present in the aqueous extract of the brain in sufficient quantity to determine their composition. Several substances however were obtained in minute quantities.

A nitrogenous body from basic acetate of lead precipitate.

A brown nitrogenous body was obtained from the basic lead acetate precipitate with uric acid, crystallising in microscopic spheres. This basic lead precipitate also contained a second nitrogenous body in small quantities.

A second body from same precipitate.

The mother liquor from the inosite, after the addition of absolute alcohol produced no further crystallisation of inosite, was neutralised with baryta water; no turbidity ensued. It was then evaporated to one quarter and treated with at least an equal volume of alcohol when a white precipitate fell consisting principally of baryum chloride mixed however with a small quantity of organic matter. The filtrate from this precipitate was evaporated to a syrup

Acid mother liquid from inosite treated

and allowed to stand, when yellowish granular and warty glistening crystals were deposited, easily soluble in water hot and cold and alcohol; blackening and burning with a luminous flame when heated on platinum foil; leaving a residue of baryum carbonate. These crystals did not consist of inosate of baryum as proved by the sulphate of copper test (a white precipitate fell but no blue color was produced). An aqueous solution of the crystals gave a voluminous white precipitate with basic acetate of lead.

deposited crystals which were not inosate of baryum.

Another nitrogenous body was obtained from the filtrate from the lead precipitate, by heating the residue in the retort, after distilling off the volatile acids and exhausting with ether, as follows: the sulphates of potassium and sodium which have crystallised out are separated off, the brown filtrate is heated with calcium hydrate boiled and filtered, the clear filtrate is concentrated on the water-bath and allowed to stand. Brown tables crystallise out soluble in water burning with smell of horn and leaving an ash of calcium carbonate.

Another nitrogenous body from residue.

Crystallises.

The mother liquor from these crystals gives with tannic acid, zinc chloride, and mercury nitrate brown floculent precipitates which however shrink so much on drying as to prevent further investigation.

Mother liquor still gives precipitates with tannic acid, zinc chloride, and mercury nitrate.

The results just sketched may be summarised thus:—

Results.

1. The brain of man in the portion soluble in water contains a small quantity of *kreatine*.
2. On the other hand kreatine is not present in ox brain, but the latter contains either *leucine or a homologue*.
3. In both human and ox brain *volatile acids* of the series $C_n H_{2n} O_2$ in very small quantities exist.
4. Both human and ox brain contain considerable quantities of *lactic acid*.
5. Ox brain contains a little *uric acid* and some quantity of *inosite*.
6. Succinic acid, glycogen, kreatinine, urea, cystine, and taurine could not be found in the brain.

In some preliminary remarks introducing his second paper, Müller refers to the difficult nature of a research on the fats, &c. of the brain, and mentions that from 30 ox brains he was only able to obtain sufficient material to establish the presence of cholesterin and cerebrin.

W. Müller's second paper. (Ann. Chem. 105 361 (1858))

The method adopted was the following:—The coagulum obtained by treating the brain matter with solution of lead acetate and heating was first extracted with hot 80 °/₀ alcohol, then boiled with a mixture of equal parts of alcohol and ether, and filtered boiling hot; on cooling a considerable quantity of a white flocculent matter was deposited, which was collected on a filter and dried. When dry it appeared as a reddish yellow crystalline mass. This coloration, on drying, was noticed by Vauquelin. Frémy attributed it to the presence of oleophosphoric acid.

Method. Coagulum by lead acetate and heat, how treated.

The reddish yellow mass was extracted with ether, until the extract was colorless, and left no residue on evaporation. A clear yellowish-red ethereal solution was thus obtained, whilst a yellowish-white voluminous insoluble residue remained. The solution contained cholesterin and a phosphorised body, having the properties of an acid, whilst the insoluble residue contained the cerebrin.

White matter extracted with ether. Cholesterin and phosphorised acid in solution. Cerebrin remains insoluble.

The ethereal solution (of the precipitate obtained on cooling the alcohol ethereal extract of the coagulum) was freed from ether by distillation. The red pasty residue, containing numerous crystals of cholesterin, was treated with alcohol, and boiled with an excess of lead hydrate. Most of the fatty acids combined with the lead, and on filtering boiling hot remained as a greyish-white powder on the filter. The filtrate, on cooling, deposited a large quantity of white shining plates, which were collected and purified by further treatment with lead, and re-crystallisation from hot alcohol.

Residue treated with alcohol and boiled with lead hydrate yielded cholesterine.

They had then the following properties:—White pearly rhombic plates, tasteless and odourless, greasy to the touch, insoluble in water, easily soluble in ether and boiling alcohol, difficultly soluble in cold alcohol. Heated on platinum foil they blackened, swelled up, and burnt with a luminous flame without leaving any residue. The substance was quite free from nitrogen and was not precipitated from its ethereal solution by reagents.

Properties.

Analysis.

Burnt with chromate of lead and a little bichromate of potash it gave the following results :—

	Theory		Found
	of equivalents	per cents.	
C 28	168	83·93	83·57
H 24	24	11·91	12·06
O	8	4·16	4·37
		100·00	100·00

This theory of cholesterin is, however, incorrect, its actual composition being (atomic notation) $C_{26} H_{44} O$.

Cholesterin forms one-third of the constituents of the brain soluble in alcohol or ether.

Cerebrin.

Müller prepares cerebrin as follows :—

Coagulum extracted with boiling alcohol.

Human brains were worked up into a thin milk with baryta water and coagulated by heating. According to Frémy baryum cerebrate is insoluble in boiling alcohol, but to Müller's astonishment on extracting the coagulum with boiling alcohol he obtained on cooling the alcoholic extract a voluminous white flocculent precipitate, which on exhaustion with ether left a yellow residue presenting after repeated crystallisations from boiling alcohol all the characteristics of pure cerebrin. In a similar way the coagulum obtained by heating the acetate of lead extract of the brain was extracted with boiling alcohol, the deposit on cooling was purified by treating with ether and taken up in boiling alcohol, when it left only a small portion of resinous matter insoluble ; on cooling a white powder was deposited which was dried and crystallised from boiling alcohol until the latter left no trace of the resinous body undissolved.

Exhausted with ether. Recrystallised from boiling alcohol. Cerebrin deposited on cooling. Same result with lead coagulum.

Properties.

The substance purified as above had the following properties :—White loose very light powder, tasteless and inodorous, soluble in boiling alcohol and ether, the solution having no reaction on vegetable colors, insoluble in water, cold alcohol, and ether. Microscopic spheres: heated on platinum-foil cerebrin gives off the smell of burnt horn becoming brown, melting, and finally burns with a red flame, leaving a black fused charcoal burning off without residue. It blackens mercurous nitrate paper when heated with soda lime. It is insoluble in ammonia, hot and cold, in potash and in baryta water. It is unchanged by cold water, swells up like starch in boiling water changing to a thin slightly turbid emulsion which remains on cooling, and is not changed by the addition of acids, alkalies, or metallic salts. If it be evaporated a residue remains soluble in hot alcohol, from which solution cerebrin is deposited on cooling unchanged.

Reactions with HCl.

HNO³.

$H_2 SO_4$.

Hydrochloric, phosphoric, and nitric acids produce no change in the cold. By boiling with hydrochloric acid the body becomes first reddish violet then it decomposes forming a brown fluid and a brown resinous body insoluble in acids and alkalies. On boiling cerebrin with nitric acid nitrous fumes are evolved, the fluid becomes yellow, and yellow oily drops form on the upper part of the flask, solidifying on cooling to a white fatty body. Concentrated sulphuric acid dissolves cerebrin in the cold with a dark purple red color; on diluting with water the colour vanishes and a yellowish white precipitate falls.

No S or P.

Decomposed at 80°.

Cerebrin is free from sulphur and phosphorus. It could not be dried at 100° C. as it decomposed at 80° becoming brownish yellow.

Analysis of cerebrin.

Analysis of the cerebrin dried at 75° C., when it lost no water, gave the following results :—

Combustions with chromate of lead and nitrogen estimated as ammonio-platinum chloride—

		Theory.		Found		
C	34	204	68·23	68·35	68·56	—
H	33	33	11·04	11·30	11·25	11·06
N	1	14	4·68	4·69	4·53	4·29
O	6	48	16·05	15·66	15·66	—

Cerebrin not cerebric acid minus the phosphorus.

Müller points out that these figures prove that his cerebrin is not identical with Frémy's cerebric acid, minus the phosphorus, because the above percentages

of carbon and hydrogen are higher than those obtained by Frémy, so that Frémy's cerebric acid was probably contaminated with some phosphorised fat.

Discussion as to the acid nature of cerebric acid.

Müller then proceeds to discuss the supposed acid nature of Frémy's body. The solubility of the supposed insoluble baryum and lead cerebrate in alcohol has already been mentioned. He treated boiling alcoholic solution of cerebric acid with sufficient potash solution and obtained a voluminous white precipitate which on further boiling partially redissolved. Filtered boiling hot nearly pure cerebrin remained on the filter. Treatment with baryta gave precisely similar results; on long boiling a yellow resinous insoluble body was obtained sticking to the sides, probably from a primary decomposition of cerebrin and combination of the products of decomposition with the alkalies. Moreover, as Frémy dealt with an impure substance, and states that it combined with the baryta, it becomes a question how far the impurity and how far the cerebric acid took part in the combination. At all events the reasons adduced by Frémy will not suffice to prove this body to be a well characterised acid; on the contrary, its behaviour towards vegetable colours and bases proves that it belongs to the class of neutral nitrogenous bodies, and if it could be combined with bases it might be said that other neutral bodies as glycin, allantoin, &c., do the same.

Is not an acid, but a neutral nitrogenous body.

Names it *cerebrin*.

The author therefore assumes the name "cerebrin" for his new substance; the cerebrin discovered in the blood fats by Gobley was probably a combination of cerebrin with a phosphorised fat.

Gobley's cerebrin.

Reactions with nitric acid.

Some cerebrin was placed in a retort with concentrated nitric acid, a receiver was attached and the retort gently heated; at first the substance somewhat swelled up, soon vapours of nitrous acid were disengaged, and a clear liquid went over into the receiver. The red fumes increased with much frothing, whilst the substance became transparent. As soon as it began to boil the substance changed suddenly into a yellow transparent oil, which floated on the yellow nitric acid, and for some long time underwent no further change. The distillation was now stopped, and the contents of the retort poured whilst hot into a beaker, the yellow oil solidified into a yellowish white solid fatty body, which was freed from nitric acid by filtration and washing; it was then dissolved in boiling alcohol and filtered boiling hot, when after 24 hours' standing a white granular fatty body separated; this fatty body was purified by recrystallisation, and obtained as a white waxy mass, soluble in alcohol and ether with moderate facility; in alcoholic solution it had a slight acid reaction.

Oily body formed.

Properties.

Under the microscope it was seen to consist of minute fat globules, without a trace of crystals. Heated on platinum foil it melted quickly to a yellowish fluid, blackened and burnt with a ruddy flame, evolving pungent odours of burnt fat.

Analysis.

On analysis it contained 12·92 °/₀ hydrogen, 75·52 °/₀ carbon, and probably no nitrogen.

	°/₀ found.	÷ by At. Wgts.	÷ O as 1.
C	75·52	6·293	8·71
H	12·92	12·920	17·89
O	11·56	0·722	1·00

$= C\ H_{18}\ O$, or $C_{17}\ H_{35}\ O_2$ (atomic notation).

Brain Fats.

Alcoholic mother liquor from cholesterin or cerebrin boiled with lead hydrate.

Precipitate treated with ether.

Ethereal extract evaporated.

Residue partly soluble in boiling alcohol.

Insoluble residue dissolved in ether.

Evaporated.

Left 20 grm. of reddish yellow body.

The alcoholic solution of the coagulum after the cholesterin and cerebrin had been separated was treated with hydrate of lead and boiled. A lead salt of a fatty acid, mixed with the excess of hydrate of lead went down. The precipitate was filtered off and exhausted with ether. A bright reddish-yellow ethereal extract was thus obtained, whilst the lead salt remained as a fine powder.

The extract on evaporation left a reddish yellow waxy mass, a small portion of which was soluble in boiling alcohol, and was obtained as a fine white powdery lead salt, decomposing and turning brown at 70°. The residue insoluble in boiling alcohol was redissolved in ether, a small quantity of a yellowish-white powder remaining undissolved; the solution was filtered, and the dark red alkaline filtrate evaporated spontaneously, leaving a considerable quantity of a hard fixed reddish brown body, which on powdering became of a reddish-yellow colour. About 20 grammes were obtained. It was not changed by further solution in ether and evaporation. Insoluble in water and alcohol,

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Properties.

No N or S.

Mixture of various bodies.

Analysis.

soluble in ether. Heated on platinum foil it blackened, melted, swelled up, gave off a peculiar smell, and burnt with a red flame, leaving behind a considerable residue of metallic lead, oxyde of lead, and pyrophosphate of lead. It contained no nitrogen and no sulphur. On analysis it was found to consist of a mixture of lead salts. The lead was estimated by fusion with nitre, dissolving the residue in nitric acid, and precipitating by sulphuric acid. It contained—

44·86	45·17 and 42·86 °/₀ lead.
29·7	29·19 and 28·26 °/₀ carbon.
4·31	4·19 hydrogen, and 3·72 °/₀ phosphorus.

Baryta salt of above prepared.

These differences are too great to be accounted for by defects in the analysis, moreover the baryta salt does not agree with the lead salt. The baryta salt was prepared by treating the lead salt with sulphuretted hydrogen suspended in water. The filtrate from the lead sulphide was yellow acid and clear. It was evaporated on the water bath to drive off the sulphuretted hydrogen, and the fluid divided into two parts, one was further evaporated on the water bath and allowed to stand. After many days standing a solid soft reddish brown sticky acid mass separated, which only partially redissolved in alcohol. The other portion was treated with baryta water, which produced a yellow flocculent precipitate, it was filtered off and dried when it became reddish-yellow, and easy to powder. Heated on platinum foil it left an ash of carbonate and phosphate of baryta. The body was insoluble in water, alcohol, and ether, and contained 32·81 °/₀ baryum.

Body not glycerophosphate or oleophosphate.

It could not be glycerophosphate, as the glycerophosphate of bargum is easily soluble in water; nor could it be oleophosphate, since the free body was soluble in alcohol, whereas oleophosphoric is only soluble in ether.

Müller thinks it safest to consider the question as to what form the phosphorus is contained in the brain fats as unsettled.

Portion of lead salts insoluble in ether.

The residue of the lead salts of the fatty acids insoluble in ether was a dirty yellow light powder, insoluble in water, alcohol, and ether. It was treated with sulphuretted hydrogen whilst suspended in water; the filtrate evaporated considerably on the water bath, and then allowed to evaporate spontaneously. After several days the residue had separated into a yellow, oily, acid layer, and many pearly-white scales; the latter were filtered off, and obtained white by recrystallisation from alcohol. Easily soluble in alcohol and ether, left no residue on ignition. The quantity was too small to purify or investigate further.

White scales.

Properties.

Conclusions.

The conclusions thus arrived at may be stated:—

1. The brain contains a very considerable quantity of cholesterin.
2. Also a considerable quantity of a nitrogenous body with the formula $C_{34}\,H_{33}\,NO_6$.
3. Also a body containing phosphorus which forms with lead a compound soluble in ether.
4. Also volatile and fixed fatty acids.

Liebreich's *Protagon.*

O. Liebreich's research on protagon was published in Ann. Chem. 134, 29.

Brain washed *in situ.*

When an animal is killed by severing the carotid arteries, and water injected into the arteries and through the brain until it leaves the veins colourless, the entire brain or the greater part of it is obtained free from blood. It may be removed from the cranial cavity and liberated from its membranes with facility, as these latter have become dropsical. The brain substance is then cut to pieces or kneaded with the hand and triturated in a mortar, and then mixed with water and ether in a bottle and shaken. The mixture is then allowed to stand at 0° C. until the ether has collected on the surface. The ether is then removed and contains besides cholesterine a very little protagon. This extraction is repeated, and has for its result that the greater part of the cholesterine is removed from the brain matter, whilst the ingredients which are easily soluble in water pass into the water.

Comminution of brain matter.

Cholesterin extracted by ether.

Treatment with alcohol.

Alcohol solution at 45° deposits flakes on cooling. Flakes washed with ether, dried, and recrystallised.

The brain matter is now freed from water and ether by filtration digested with spirit of 85 °/₀ strength, in a water bath at 45° C., and the alcoholic solution filtered off on a hot funnel kept also at 45° C. This extract is then cooled to 0° C., when it deposits a copious flaky precipitate. This precipitate is collected on a filter and washed with cold ether until no cholesterine is extracted

by the ether. It is then dried in vacuo over sulphuric acid, afterwards moistened with a little water and dissolved in spirit at the temperature of 45° C. This saturated solution is again filtered at 45°, and then allowed to cool gradually in a large water bath to the temperature of 16° C. Minute crystals are deposited, visible as crystals only under the microscope. If the solution had the proper degree of concentration these crystals assumed the form of needles in radiary arrangement. In very concentrated solutions the crystals are bent and distorted. They can be recrystallised until pure.

Crystals obtained by gradual cooling.

Purified by recrystallisation.

Another method of obtaining protagon is the following. The brain is triturated with water and ether, and allowed to stand for some time at the ordinary temperature. The ether absorbs fatty acids produced by the decomposition of a portion of the protagon and much protagon which is easily soluble in the products of its decomposition. The mixture is heated to 29° C., the ether separated off, filtered, and cooled down to a very low temperature. The protagon separates as a white precipitate, which is placed on a filter, washed with cold ether to remove cholesterine, dried, and recrystallised from alcohol as described above.

Brain extracted with water and ether.

Ether extract cooled to 0° C.

Protagon deposited.

Protagon thus prepared gave following analyses and theory leading to formula $C_{116}\ H_{24}\ N_4\ O_{22}\ P$.

Mean of analyses

	Theory. Atoms.	Theory. %	Mean found
C_{116}		67·21	66·74
H_{241}		11·59	11·74
N_4		2·7	2·8
P_1		1·5	1·23
O_{22}		17·0	17·49
		100·00	100·00

The carbon was usually a little low and hydrogen high. The phosphorus varied from 1·1 in ox to 1·5 in human brain.

(The hydrogen, to make an even number with the nitrogen, must be either 240 or 242).

Protagon recrystallised from alcohol and dried in vacuo over sulphuric acid is a light flocculent powder. The crystals obtained from dilute alcohol assume a wax-like appearance before they part with all their water.

Peculiarities of protagon.

In cold alcohol and cold ether the substance is but little soluble, more soluble in proportion as these solvents are warm. In absolute alcohol, protagon cannot be heated above 55° C. without decomposing; a solution which has been thus treated, exhibits oily drops, and on cooling, deposits round balls, besides crystals, denoting that a decomposition has taken place. Treated with water, protagon swells, and becomes an opaque mass, resembling flour paste. By dilution with more water, a clear but opalescent solution is obtained. When the watery solution is mixed with concentrated solutions of salts, such as calcium chloride, sodium chloride, &c., a coagulation ensues. The flakes of protagon which float about the fluid may be filtered off; the filtrate seems to be free from protagon. The precipitate is not a chemical compound, for on the filter the salts can be washed away, and during washing the protagon swells up again. But it is difficult to obtain it quite pure without great loss, as the purer it becomes, the easier does it pass through the filter. In glacial acetic acid protagon dissolves, giving a clear solution, which on slow cooling, furnishes crystals similar to those obtained from alcohol. Protagon decomposes when submitted to a temperature below 100° C. The dryer the substance is, the more quickly does it decompose. At a temperature 75°–80° C., the flaky matter becomes soft, and may be kneaded together. Protagon which has been boiled, and thus swollen with water, may be dried in vacuo over sulphuric acid, and on solution in alcohol, the whole of the original matter crystallises out on cooling, unchanged. When heated more strongly, protagon fuses, becomes brown, burns with a yellow sooty flame, and leaves a black dense charcoal, which has an acid reaction when moistened with water, from the presence of phosphoric acid. This latter substance also prevents the ready combustion of the carbonate.

Solubility in ether and alcohol.

Behaviour with water and saline solutions.

Soluble in glacial acetic acid.

Influence of heat.

App. No. 5.

On the Chemical Constitution of the Brain, by Dr. Thudichum.

Decompositions of protagon. Decomposition with baryta water.

Protagon is boiled with concentrated baryta water for 24 hours. The excess of baryta is then precipitated by carbonic acid; the precipitate filtered off. The filtrate contains glycerophosphoric acid combined with baryum and a new base neurine. The baryta salts of several fatty acids remain with the baryum carbonate. The entire filtrate is concentrated on the water bath at a moderate temperature, and precipitated with basic lead acetate. This precipitate contains the glycerophosphoric acid; the filtrate from it contains the neurine. The lead precipitate is suspended in water, and decomposed with hydrothion yielding a strongly acid fluid. This is filtered from the lead sulphide, saturated with calcium carbonate, and concentrated; it deposits while hot the characteristic scales of calcium glycerophosphate.

Glycerophosphate and neurine formed and separated.

Calcium glycerophosphate.

Baryum glycerophosphate.

The baryum salt may be obtained by concentrating the watery filtrate from the protagon after treatment with excess of baryta water, at a moderate temperature, and ultimately in vacuo over sulphuric acid, removing any carbonate or phosphate of baryum which may be precipitated. A transparent glass-like mass is thus obtained, which is very hygroscopic. It is extracted with absolute alcohol as long as anything (neurine) is extracted. The baryum glycerophosphate remains as a white powder, easily soluble in water. In the *aqueous* solutions of baryum or calcium glycerophosphate the presence of phosphoric acid cannot be proved by the ordinary tests for that acid; it can only be shown after calcining the salts. When the salts are treated with acid sodium sulphate the characteristic odour of acrolein is evolved.

Reactions of glycerophosphate.

Fatty acid obtained from protagon.

The baryum salts of the fatty acids remain with the baryum carbonate in the residue, from which the solution of glycerophosphate and neurine has been filtered. The following steps may be taken to effect their separation:—

The entire residue is treated with dilute sulphuric acid, and then agitated in a bottle with ether. The ether containing the fatty acids is evaporated; the residue dissolved in alcohol and precipitated with an alcoholic solution of lead acetate. The dried lead soaps are repeatedly treated with ether; there remains a lead soap insoluble in ether, which is dissolved in alcohol and treated with sodium carbonate to obtain the soda soap. This, dissolved in alcohol, is precipitated fractionally by baryum chloride. The second and third precipitates contain 19·08 and 18·7 % of Ba. The acid contained in the first salt after separation fused at 57·5° C.: that of the second salt fused at 52·5° C.

Stearic acid.

New acid.

The fatty acid seems to be impure stearic acid. The impurity seems to be an acid the lead soap of which is soluble in ether. It does not seem to be either palmitic or oleic acid, but a new hitherto unknown acid. When it is obtained in the free state after the manner prescribed by Gottlieb for oleic acid, it appears as a white gelatinous vibrating mass, which can be obtained crystallised in little needles from its alcoholic and ethereal solution. It contains neither nitrogen nor phosphorus. At higher temperatures it decomposes, yielding fluid acids.

Decomposition of protagon by hydrochloric acid.

When protagon is boiled for 12 hours in very dilute hydrochloric acid in the dark, white flakes and a yellowish fluid result. If these flakes be placed on a filter and washed until the water passes through opalescent, they resemble much the swollen protagon, but are much more transparent. Dissolved in alcohol and crystallised slowly, this matter deposits needles very similar to those of protagon. The alcoholic solution, when exposed to the rays of the sun, is decomposed, depositing a reddish brown powder, and assuming a reddish colour. The crystals contain no phosphorus. When more concentrated, hydrochloric acid is employed, a decomposition ensues which results in the production of smeary matters.

H. Koehler.

Koehler's research was published in the form of a dissertation, entitled:

De Myelini quod vocant constitutione chemica disqu. Halae 1867. (German version in Virchow's Archiv. Also more full and last publication: Chem. Unters. über die fälschlich Hirnfette genannten Substanzen und ihre Zersetzungs-producte. Halle, 1868.)

First alcohol watery extract.

Lead precipitate.

The author hardened the brain as much as possible in alcohol at 35 to 45°, and thereby obtained a *first alcoholic watery extract*. From this he obtained, by various operations, formic acid; a volatile fatty acid containing more than 8 C and H atoms; lactic acid, inosite, hypoxanthine, kreatine (if the brain was human), albumen; cholesterine, but no glycerophosphoric or stearic acid, and no neurine. Several of these matters were obtained by the agency of lead acetate

added to the solution, which produced a copious precipitate. Two cows' brains gave 248 grains of this Pb precipitate. The precipitate was insoluble in water, ether, and alcohol, and could be boiled with these agents without apparent change. It was suspended in water, and treated with hydrothion; the mixture filtered and the sulphide of lead extracted with boiling water. The extracts were evaporated to a syrup, and put in a cool place for some days. Crystals formed in the syrup, but could not be separated. Therefore, the whole was dissolved in boiling water, and mixed with four volumes of absolute alcohol. A white, powdery, yet adhesive, precipitate fell, which was recognised as hypoxanthine. The solution on standing deposited inosite.

Hypoxanthine inosite.

The solid brain matter which had thus been hardened in alcohol, and yielded to it some of its more soluble matters, was now exhausted with ether, in an apparatus surrounded with ice. The yellow, clear, transparent, ethereal extract was concentrated by distillation at 40° to one quarter, and then precipitated by absolute alcohol added gradually, until a filtered sample was not any longer made turbid by more alcohol. A white dhesive neutral substance was deposited, which the author termed *myeloidine*.

Ether extract.

Precipitation by alcohol.

It will be seen that this process is the same as that of Couerbe, and that thus far myeloidine is nothing but the cephalote of Couerbe.

The myeloidine was washed with alcohol, and dissolved in water; the solution was filtered through a bag, and required constant stirring to get it through its meshes. The cholesterine contained in the myeloidine mostly remained in the bag. To the thick turbid filtrate neutral lead acetate was now added, which produced a copious precipitate. This was extracted with alcohol at 30° to 35° C, and dried over $H_2 S O_4$. It was next digested with ether, which extracted cholesterine and *myeloidinate of lead* (to be described below), it was further extracted with boiling ether. There remained a white salt insoluble in cold and boiling alcohol, and ether, which dried at 100° in the air bath, was analysed.

Myeloidine.

Purification.

Lead salt

Analyses.

C	48·11	49·66	—
H	7·56	7·65	—
N	1·77	1·55	—
P	3·59	2·91	2·29
Pb	22·02	21·81	20·37
O	16·95	16·42	—

Leading to formula $C_{40} H_{76} N P Pb O_{10}$.

Formula.

The free myeloidine was obtained from this lead salt by suspension in ether, and treatment with dry hydrothion until a small portion of the lead salt remained undecomposed. (The wording suggests that an excess of hydrothion might have to be avoided.) The ruby-red solution was filtered from the sulphide of lead and mixed with alcohol, when it produced a white reddish precipitate, which was dried in vacuo, but apparently not analysed.

Free myeloidine by decomposition of Pb salt by $H^2 S$ in ether.

Or the myeloidine lead was suspended in water, treated with hydrothion, and the myeloidine extracted with ether.

By hydrothion in water.

The watery solution of myeloidine gave precipitates with tannin and metallic salts.

The Alcoholo-ethereal Mother Liquor, filtered from the myeloidine, was precipitated with hot alcoholic neutral lead acetate, and yielded a copious deposit. The precipitate was filtered, washed with water, afterwards absolute alcohol, and dried in vacuo over sulphuric acid. It was a yellow, loose, easily powdered salt. On being digested with ether it dissolved, leaving a little myeloidine lead undissolved. The solution was freed from ether, partly by distillation, partly in vacuo; and this resolution in ether was repeated until the solution remained quite clear, when it was precipitated by absolute alcohol. It was found to have the empirical formula $C_{74} H_{135} Pb_5 N_2 P O_{25}$, as derived from the following analyses:

Mother liquor.

New lead salt soluble in ether.

Formula.

				Mean.
C	35·42	35·71	35·49	35·54
H	5·52	5·63	5·41	5·52
N	1·15	—	—	1·15
P	1·06	1·13	—	1·09
Pb	39·42	40·89	—	40·15
O	—	—	—	16·55

Analyses.

Myeloidinate of lead.

The phosphorus was determined by the method of Carius. The salt, suspended in alcohol, was decomposed by hydrothion, until a minimum remained undecomposed, because, as the author says, an excess of hydrothion was hurtful. After evaporation of the filtrate from the sulphide of lead in vacuo, there remained a body free from lead, tallow-like, yellowish; fusing at 30°, fluid at 34°; soluble in ether, alcohol, and fatty oils, and having an acid reaction. This was termed *myeloidinic acid*. The theory of the free acid, after deduction of lead, is as follows:

Analysis of Myeloidinic acid.

	°/₀ in 100 of organic matter.
C	59·38
H	9·22
N	1·92
P	1·82
O	27·65
	99·99

Properties and reactions of its alcoholic solution.

The acid was insoluble in cold water, swelled at 45°; on long boiling in water it decomposed and dissolved. Its alcoholic solution was precipitated in white flakes by alcoholic ammonia; the same solution produced the original precipitate with lead acetate. Basic copper acetate produced a precipitate; tannin a yellow precipitate, insoluble at 100°. Concentrated sulphuric acid produced a turbidity; the mixture after evaporation of alcohol left an oily body. Alcoholic baryum acetate produced no precipitate in its alcoholic solution. Myeloidinic acid is decomposed by hydrochloric acid; when separated by this acid from lead salt it appears in the shape of swelled lumps.

The author leaves it doubtful whether this acid is already formed in the brain, or produced by the chemical operations from a neutral substance.

Neurolic acid is stated by Köhler to be a decomposition or metamorphic product of myeloidine. It is obtained:—

Neurolic acid.

First process.—Myeloidine lead is suspended in ether, and decomposed by hydrothion, and the mixture is evaporated. Alcohol is added, which leaves sulphide of lead behind on filtration (from this it appears that the free myeloidine is soluble in (hot?) alcohol T). The solution is again evaporated with water, and then leaves an oil and flakes. Or myeloidine lead is suspended in alcohol, treated with hydrothion, and the filtrate evaporated to a syrupy consistence on the water bath.

Second process.—The ethereal extract of the brain is evaporated, and the residue boiled during three to four hours with alcohol of 85 °/₀ strength; the extract is allowed to cool and filtered; the filtrate is precipitated by alcoholic lead acetate solution, and precipitate filtered off; the fluid (!) is now treated with hydrothion, and, after addition of some solid baryum hydrate, is evaporated to a syrupy consistence. After cooling the crystals, which consist of cholesterine and baryum acetate, are pressed between blotting paper; the syrup which creeps into the paper is dissolved in very little cold alcohol, precipitated with alcoholic solution of sublimate, and the precipitate dried in vacuo. It contains yet a little cholesterine. To remove this the precipitate is suspended in alcohol and decomposed with hydrothion; the cholesterine then crystallises out.

Properties of neurolic acid.

Neurolic acid is a viscid liquid, reddish, similar to ear-wax, and does not become solid at 12°. It is soluble in water, alcohol, ether, ethereal and fatty oils, even in the cold. The solutions have an acid reaction, and are easily filtered. When warmed with water on the water-bath it decomposes, and after evaporation of all water a residue of grey flakes remains, which become violet under the influence of sulphuric acid and iodine. Continued boiling with caustic potash or baryta water decomposes neurolic acid. Acetic and dilute mineral acids do not affect neurolic acid; by concentrated acids it is destroyed; concentrated sulphuric acid changes it into a red oily viscid mass. The alcoholic or watery solutions of neurolic acid are made turbid by addition of the acetates of ammonia, lead, copper, baryum, zinc, and of chloride of iron; they are, however, precipitated by both sublimate and tannin; the mercury

precipitate is white, the tannin precipitate yellow; both are voluminous and insoluble on boiling.

Calculated formula $C_{50}\,H_{90}\,P\,O_{17}$.

C	66–60·9
H	9·81–9·9–9·97
P	2·97

Another compound obtained by Koehler is erythrostearic acid with lead = $C_{36}\,H_{70}\,N\,P_2\,Pb_5\,O_{20}$. It is soluble in ether, but is still less defined than the foregoing very doubtful products, which do not appear to me to admit of any approach to identification.

Erythrostearic acid.

Myelomargarine.—The brain substance, which had been exhausted as above with cold ether, was now boiled during two hours with alcohol; the filtrate was allowed to cool and deposit, and the precipitated white matter was completely freed from cholesterine by washing with ether. The matter was now boiled with alcohol *containing sulphuric acid* (*!*) and filtered boiling; calcium and sodium sulphates remained on the filter, and the solution on cooling and standing again deposited the white matter. This was again filtered, digested with cold ether, dried in vacuo, dissolved again in alcohol, and the alcoholic boiling solution filtered into alcohol containing ammonia. A powdery completely white ammonia salt fell, which was isolated and dried, stirred with water, decomposed by hydrochloric acid, washed with water, dried, washed with cold ether, and ultimately repeatedly crystallised from hot ether.

Myelomargarine obtained and purified.

Characters:—Free from nitrogen and phosphorus.

Formula:—$C_{16}\,H_{36}\,O_5$ or $C_{16}\,H_{34}\,O_4$, $H_2\,O$.

Analysis.

	Required.	Found.
C	63·75	64·05–63·85
H	11·25	11·21–11·09
O	25·00	
	100·00	

Properties.

White soft powder, fatty to touch, soluble on warming in water, alcohol, ether, ethereal and fatty oils, separating from the solution on cooling. Not changed at 150°, becoming yellow above 150°, at 185° reddish without fusing. Heated on platinum, fuses to a reddish oil, burns with a lighting flame, and leaves an easily consumed charcoal.

The solutions of myelomargarine are neutral, and are precipitated only by basic acetate of lead (not by alcoholic solution of neutral acetate, which the author says is an important distinction from myeloidine, which is so precipitated): [and from the cerebrine-bodies, which are also so precipitated.—T.] its alcoholic solution is further precipitated by basic copper acetate and platinic chloride; the latter precipitate is soluble on boiling. The other precipitates are insoluble in hot water. Other mineral salts give no precipitates. Tannin also gives a precipitate which is insoluble in hot water. Concentrated mineral acids destroy myelo-margarine. Potash, ammonia, and baryta water form with it insoluble compounds. When mixed with cholesterine it yields the microscopic forms termed *myeline*.

From these data it is evident that Koehler completely failed in isolating any of the immediate principles of the brain which I have described as the cerebrines, phrenosines, and kerasines. Cerebrine contains nitrogen, fuses and blackens below 100°, and is in many other respects so different from myelomargarine that the two bodies could not have been mistaken for each other. But it is probable that Koehler decomposed the cerebrine and other bodies by boiling with sulphuric acid in alcohol, and that his substance is a metamorphic product.

The next investigation we have to notice is by Otto (Chem. Centr. Bl. 12 1022, 1867). Ox brain was rubbed with water to a thin paste, pressed through a cloth, mixed with excess of lead salt, after 12 hours' standing rubbed through a sieve and boiled. The coagulum was pressed, repeatedly treated with hot alcohol, and pressed; the alcoholic extracts, after cooling, were

Otto's cerebrine. Müller's process.

decanted from the precipitate, and the latter exhausted with ether. The matter insoluble in ether was again dissolved in hot alcohol; the deposit which had formed after 24 hours was again dissolved and mixed with baryta water, the fluid was filtered hot from the plaster-like precipitate, and on cooling deposited cerebrine as a transparent mass, which was first dried over H_2SO_4, next at 50° to 70°. This cerebrine contained no ash, *no nitrogen* nor phosphorus, and on analysis gave—

Otto's analyses.

C	67·2	67·6	67·8	67·8	Mean 67·6
H	11·1	11·3	10·9	11·1	„ 11·1
O	-	-	-	-	- 21·3
					100·0

These quantities divided by the atomic weights lead to

Calculation by Th.

C	5·63 × 3	= 16·89
H	11·10 „ „ „	33·30
O	1·33 „ „ „	3·99

Divided by oxygen as one,

O	1 × 4	= 4·00
C	4·23 „ „ „	16·92
H	8·35 „ „ „	33·40 = to $C_{17}H_{33}O_4$.

Formula.

Mueller's cerebrin, $C_{17}H_{33}NO_3$.
Koehler's myelomargarine, $C_{16}H_{36}O_5 = C_{16}H_{34}O_4 + H_2O$.

Hot water.

Heated in a sealed tube with water to 130°, it became a pasty mass. This again dried at 100°, was entirely soluble in absolute alcohol, and deposited in the original form and composition, analysis giving C 67·2, H 11·3 %. Heated during 12 hours with hydrochloric acid, it yielded a matter similar to fat, soluble in ether and alcohol, and deposited from the alcoholic solution as a gelatinous mass, which contained 71·2 % C and 12·2 % H. The mother liquor contained a body which fused at 66°, and contained 81·3 % C and 13·3 % H. The hydrochloric acid fluid, separated from the fat-like matters, was evaporated over H_2SO_4 and caustic potash, and yielded a syrup which could not be obtained colourless, showing traces of crystals; reduced alkaline copper solution, was precipitated by basic lead acetate (this precipitate became red on warming), and contained 41·7 % C and 7·32 % H. Koehler examined some cerebrine prepared by Otto, and reports it to be easily soluble in the cold in alcohol, ether, and turpentine; in fatty oils, chloroform, benzine, and glycerine soluble only on boiling; deposited on cooling from benzine and glycerine. Fuses a little above 150° and becomes yellow, after which it does not swell any more with water. Neutral reaction. With H_2SO_4 gives violet solution, and is destroyed. Dissolves in concentrated HNO_3, without red fumes. HCl acts slowly, acetic acid not at all. Its alcoholic solution is precipitated by picrino-nitric acid, yellow; by silver nitrate in white flakes, which slowly darken. Both these precipitates are insoluble on boiling, and bake together. Basic copper acetate, bluish white; stannous chloride, white; both the latter insoluble on boiling. Palladium chloride, white flaky precipitate, soluble on boiling. No precipitates are obtained by the acetates of baryum, mercury, and zinc, or by iron salts.

Hot HCl.

Koehler tests some of Otto's preparations.

Diakonow on lecithin.

The next researches on the phosphorised matters were made by Diakonow (Centr. bl. No. 1, 1868).—Pure lecithin is a yellowish white waxy, in thin layers, silky, highly hygroscopic matter, soluble in ether and alcohol, swells in water, and when shaken with it forms a wax-like paste. Heated, it burns completely, leaving phosphoric acid. It contains—

C	64·27 %
H	11·4 %
N	1·8 %
P	3·8 %

These numbers lead to the formula $C_{44}H_{90}NPO_9$ + aq.

When heated in a solution of caustic baryta, stearate of baryum is given off in granules at the moment of ebullition, and the clear fluid contains glycerophosphate of baryum and trimethyl-oxaethylammonium oxyde hydrate (neurine). These are the only three products of decomposition—

Glycerophosphoric Acid. Stearic Acid. Neurine. Lecithine.

$$C_3 H_9 P O_6 + 2(C_{18} H_{36} O_2) + C_5 H_{15} N O_2 - 3 O H_2 = C_{44} H_{90} N P O$$

Type.—Glycerophosphoric acid is an anhydrohydrate, with four extra-radical replaceable H atoms— Type.

$$\left.\begin{matrix} H_2 \\ C_3 H_5 \\ P O \\ H_2 \end{matrix}\right\} \begin{matrix} O_2 \\ O \\ O_2 \end{matrix}$$

The other two hydrogen atoms have remained from the phosphoric acid, and are therefore easily replaced by bases. In placing, in lieu of the first two hydrogen atoms in glycerophosphoric acid, the radical stearic acid twice, and once the radical of neurine in lieu of one hydrogen, we obtain—

$$\left.\begin{matrix} 2(C_{18} H_{35} O_2) \\ C_3 H_5 \\ P O \\ C_5 H_{14} N O \\ H \end{matrix}\right\} \begin{matrix} O_2 \\ O \\ O_2 \end{matrix} \Bigg\} = C_{44} H_{90} N P O_9$$

This compound must yield by caustic alkali stearate, and glycerophosphate and neurine. Lecithin, therefore, is a compound of an acid ether (the glyceride distearine), with an acid salt (acid phosphate of trimethyloxaethylammonium), forming an anhydride molecule. Its name might be Distearyl-glyceryl-phosphate of trimethyloxaethylammonium.

In a subsequent paper (Centr-bl. No. 7, 1868) Diakonow makes more explicit statements, and describes his mode of preparing the body, which was omitted in the first communication.

Brain (cow) was extracted repeatedly with ether, the residual matter treated with alcohol at 40°, and the alcohol extract cooled to 0°; the precipitate which formed was filtered off, washed with a little ether and cold absolute alcohol, and again extracted with ether; a portion of the matter dissolved in ether, another remained insoluble (Protagon); this was repeatedly extracted with ether at the ordinary temperature; from all these extracts the ether was distilled off; the residue dried at 40° and dissolved in a minimum of absolute alcohol. On cooling the solution to 7° a white substance was deposited, which had the composition and properties of lecithin. Diakonow on lecithine in brain.

Amorphous not pulverisable, hygroscopic, swelling in water; forming an emulsion with it on shaking; leaves when burned phosphoric anhydride. Boiled with alkaline earths yields gycerophosphate and stearate, with neurine.

It yielded $P_2 O_5$ 7·83 °/₀ – 7·89°/₀.
N 1·85 °/₀.

Lecithine $C_{44} H_{90} N P O_9$ and aq. requires
$P_2 O_5$ 8·378 °/₀.
N 1·71 °/₀.

Protagon is declared to be a body free from phosphorus, containing lecithin e. Lecithine is easily precipitated from solutions in which other precipitates form For this reason the cholesterin from brain, and the lactates from muscles, contain phosphorus. The crystalline precipitates, containing phosphorus, which Hermann obtained from blood corpuscles, Fischer from pus, Kühne from extract of eggs, are declared to be protagon. Köhler's myeloidin and myeloidinic acid are all but impurities mixed with lecithin. So says Diakonow.

Protagon should yield 3·428 °/₀ $P_2 O_5$ while after exhaustion it yields 2·34 °/₀. Re-crystallised from warm alcohol it loses still more phosphorus; a

body passes into the alcohol which, burned with nitre and soda, yields 7·4 % $P_2 O_5$. Protagon, treated with much warm water, and extracted with ether, lost phosphorus until it yielded only 1·5 % $P_2 O_5$.

Protagon boiled with baryta water, and the white precipitate extracted with hot alcohol, yields a great quantity of a matter which is deposited on cooling. This is neutral, swells in hot water, dissolves in hot alcohol and ether, but not in cold. On burning it leaves no ash, unless too much alcohol had been used, and boiling continued too long. Further boiling with baryta water does not change the matter. Treated with sulphuric acid, it reduces copperoxyde. The white precipitate contains a baryum soap and glycerophosphate; it is therefore clear that of the protagon only the lecithin is decomposed, while the glucoside remains unchanged. In this way it is intelligible how Liebreich could once obtain sugar. Frémy and Bibra described the body; Müller has described the body, obtained it pure, and termed it Cerebrin.

Lecithine from eggs: Strecker.

The following research, though made on eggs, bears upon our subject.

Strecker (Sitz. Ber. d. Acad. d. W. München, 1869, 2, 269), after some allusion to previous authors, described the manner in which he isolated lecithine from yelks of eggs. He treated them with a mixture of alcohol and ether, distilled the ether off, added spirit as long as oil continued to be precipitated, and then an alcoholic solution of Pt Cl_4 acidulated with H Cl. A precipitate of lecithine chloride + Pt Cl_4 ensued, soluble in ether, chloroform, sulphide of carbon, and benzole. It was purified by solution in ether, and precipitation with alcohol. Dried in vacuo over $H_2 S O_4$; at 100° it blackened and lost weight to 5·1 %. Cadmium-chloride gave a similar precipitate, little soluble in alcohol or ether, or their mixture, easily soluble in alcohol and H Cl. According to Strecker the metals can be easily removed from the solutions of these compounds by hydrothion. The solution filtered from the sulphide being treated with a current of $C O_2$ and evaporated, leaves lecithine hydrochlorate as a waxy mass. The H Cl can be removed by silver oxyde; the excess of the latter by $H_2 S$.

Formula of platinum salt.

By boiling baryta water, a spirituous solution of lecithine hydrochlorate is decomposed, and yields baryum salts of fatty acids, of glycerophosphoric acid, and choline hydrochlorate. The Pt salt has the formula $C_{42} H_{83} N P O_8 Cl$ pt Cl_2. Carbon and hydrogen were found generally higher; platinum and chlorine much lower than corresponds to this formula. The cadmium chloride salt did not admit of a definite formula, its cadmium varying between 13 and 15 %.

Theoretical formula of lecithine.

The formula $C_{42} H_{84} N P O_9$ Strecker derives, as follows, from the decomposition products:—

$$\underbrace{C_3 H_9 P O_6}_{\text{Glycerophosphoric acid.}} + \underbrace{C_5 H_{15} N O_2}_{\text{Cholin.}} + \underbrace{C_{18} H_{34} O_2}_{\text{Oleic acid.}} + \underbrace{C_{16} H_{32} O_2}_{\text{Margaric acid.}} - 3 H_2 O = \underbrace{C_{42} H_{84} N P O_9}_{\text{Lecithine.}}$$

Lecithine combines with pt Cl_2 H Cl and with Cd Cl_2 like organic bases; but also with oxydes of metals, as silver oxide ($Ag_2 O$). Its solution in ether-alcohol gives a crystalline precipitate with alcoholic potash solution. It is therefore a base, an acid, and a fat. To prove this by analogies Strecker enters into some theoretical considerations which are of interest, though of subordinate value for the present purpose.

In this research it is rather assumed than proved that the phosphorised body of brain-matter is identical with that from eggs. On the other hand, it is certain that the great bulk of phosphorised bodies from the brain is not identical with lecithine, as their metallo-chlorides observe an entirely different bearing with hydrothion from that which Strecker describes for his lecithine metallo chlorides.

The principal progress in this research of Strecker concerns the introduction as isolating agents of the chlorides of platinum and cadmium; but the details

were insufficiently elaborated, and the cohesion of the parts was rather guessed than proved.

Baeyer. Combination with phosphorowolframic acid does not entirely purify.

Baeyer produced (Ann. Chem. 140, 306), neurine by Liebreich's process, purified it by adding to its hydrochloric acid solution phosphorowolframic acid; the precipitate which fell was washed, and decomposed with baryta water, and the resulting liquid, after removal of the baryta and addition of hydrochloric acid, was evaporated to a syrup. The hydrochlorate of neurine thus obtained is yet coloured, even though by fractional precipitation with phosphorowolframic acid the impurities which fall down with the first precipitates have been removed.

Purify by Pt Cl4 combination. Three bases.

The best method of purification consists in transforming it into the platinic compound. Several analyses of different preparations led to discordant results, indicating substances of the following formulæ:—

1. $N C_5 H_{14} O Cl, pt Cl_2$
2. $N C_5 H_{12} Cl, pt Cl_2$
3. $N C_5 H_{14} Cl, pt Cl_2$

(The small pt indicates an equivalent, $= \frac{1}{2}$ atom of Pt.)

Two bases certain and one problemetical.

A special examination of neurine yielded as a result that it is probably a mixture of two different bases, the platinic compounds of which have the compositions of the above salts 1 and 2. It is questionable whether the third base also occurs in neurine; the analysis of a preparation speaks for the assumption, as also the circumstance that the hydrogen in No. 2 is always found somewhat too high.

Neurine and hydriodic acid.

When a most concentrated solution of neurine hydrochlorate is mixed with several volumes of concentrated hydriodic acid and a little phosphorus and heated in a sealed tube to 120–150° during some hours, then after cooling the tube a considerable quantity of large colourless prismatic crystals is obtained. After recrystallisation from hot water these crystals gave analytical results leading to the formula $NC_5 H_{13} I_2$.

	Found.	Calculated.
C	17·65	17·59
H	3·94	3 81
N	4·10	4·10
I	74·26	74·48

Properties

Easily soluble in hot water, difficultly soluble in cold, crystallising on cooling in heavy, shining, imperfectly-formed crystals, which are somewhat similar to potassium iodide. From their solution caustic potash precipitates white flakes, which become crystalline. The iodine is contained in this compound in two forms; nitrate of silver precipitates only one iodine from the solution in water, whilst a base containing iodine remains in solution. On long heating, however, the second iodine is also precipitated.

Removal of one atom of iodine by Ag N O3.

Same with Ag Cl.

Freshly precipitated chloride of silver digested in the cold with the watery solution of the compounds replaces only one iodine by chlorine. The liquid filtered from the silver iodide, on addition of platinic chloride, yields a heavy yellow precipitate, consisting of small octohedric crystals. They are but little soluble in water, and have the formula

$N C_5 H_{13} I Cl pt Cl_2$.

	Found.	Calculated.
C	14·4	14·32
H	3·2	3·10
I Cl	56·4	55·08
pt	23·5	23·50

This compound, therefore, contains an iodised base.

Digested warm with freshly precipitated silver oxyde, the iodide loses both atoms of iodine, and yields a base, which treated with hydrochloric acid and

App. No. 5.

On the Chemical Constitution of the Brain, by Dr. Thudichum.

platinic chloride gives a very soluble compound, and in all respects like the neurine compound. On analysis, however, it constantly yields results corresponding to the formula $N_2 C_{10} H_{26} O Cl_2, pt_2 Cl_4$.

	Found.	Calculated.
C	19·97	20·00
H	4·49	4·33
pt	32·84 to 32·64	32·83

A. W. Hofmann obtained, by treating a watery or alcoholic solution of trimethylamine with ethylene bromide by a simple combination of both bodies a substance $N C_5 H_{13} Br_2$. This, by treating with nitrate of silver, loses one bromine, the filtrate gives with $pt Cl_2 = N C_5 H_{13} Br Cl, pt Cl_2$, difficultly soluble in cold water; oxyde of silver takes out both atoms of bromine, and the solution is alkaline, and contains neurine. Thus this compound is analogous to the above No. I compound.

Now, whereas neurine treated with hydriodic acid yields the compound $N C_5 H_{13} I_2$, which is really trimethyl-iodoethyl-ammonium iodide $(N (C H_3)_3 (C_2 H_4 I) I$, neurine must be either trimethyl-vinyl-ammonium-oxydehydrate, $(N (C H_3)_3 (C_2 H_3) HO$, or trimethyl-oxethyl-ammonium-oxydehydrate, $N (C H_3)_3 (C_2 H_4 (H O)) H O$. As the analyses of the platinum salts do not agree, it is probable that they are mixtures of both bases, which can yield the combination $N C_5 H_{13} I_2$ either by directly taking up H I or by separating water. Free neurine dissolved in water easily decomposes, yielding trimethylamine. It is therefore to be expected that some of the trimethylamine found in animals and plants during life and after death may be due to a decomposition of neurine.

The group oxethyl also occurs in taurine.

Choline from bile seems identical with one of the bases from neurine.

A. Clauss and C. Kessé on neurine and sinkaline.

The following observations prove that a base derived by decomposition from an ingredient of mustard, sinapine, is identical with the base derived from brain matter. They were made by A. Clauss and C. Kessé. (Journ. pr. Chem. 102 (1867) 24.)

Sinkaline was discovered by Von Babo and Hirschbrunn (Ann. Chem. 84, 10) as a product of decomposition of sinapine. This base the authors only saw, as prepared by the discoverers, but did not themselves prepare it. They made, however, a quantity of neurine.

The brain, after being stirred and comminuted with water, was extracted with a mixture of about equal volumes of alcohol and ether, because it was found that this solvent separated quicker from the brain matter after stirring with water than pure ether, and further, because they believe that ether alone yields ultimately less neurine (*i.e.*, dissolves less myeline and lecithin). The alcoholo-ethereal extract was immediately mixed with concentrated baryta water, and distilled during 24 hours. This process yielded more neurine from eight calves brains than Dybkowsky's process from 18. The watery solution was then treated with carbonic acid, the filtrate evaporated to a syrup, the latter exhausted with alcohol; the alcoholic solution was acidified with hydrochloric acid, and mixed with alcoholic solution of platinum chloride. A copious yellow precipitate fell in cheesy lumps. (The addition of ether to the mother liquor causes a further precipitate according to Dybkowsky, but according to the authors this (ether produced, Th.) precipitate contains little or nothing of the neurine compound). The precipitate is almost completely soluble in water and on slow evaporation of the water a mass is obtained which is more or less crystalline, and seems essentially to consist of three different substances. The platinic salt of neurine is present in quantity, and is separated from the two other bodies by fractional solution, one body being easily soluble in cold water, the other almost insoluble in it (so that platinic neurine seems to have an intermediate solubility, Th.).

The body nearly insoluble in cold water dissolves in water on long boiling, and is again deposited on cooling. Though it contains carbon, visible on combustion, the authors suspect it to be potassium or ammonium platinic chloride.

The neurine-platinic chloride dissolves in cold water, but not easily, quickly however in warm water, and is obtained from the solution in different forms. On cooling a hot saturated solution small needles are at first deposited; on evaporating the mother liquor over sulphuric acid thick columnar crystals are formed, and ultimately on exsiccation a mass of confused crystals is obtained which resembles nitrate of urea. If a small quantity of free platinic chloride be added to the solution rhombic prisms are formed.

The auric chloride of neurine (and sinkaline) crystallises from hot water on cooling in yellow needles, later on in yellow scales. On heating the salt the smell of trimethylamine is observed.

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